

British universities rebel

After a decade in which their resources and independence have been whittled away, British universities have uttered a cautious act of defiance. The government will be foolish if it tries to bring them to heel.

ONE of the best-known techniques of civil disobedience is for the disobedient to insist that they will do what is required of them only if compelled by law. From British India to Brezhnev's Soviet Union, there is ample proof that the technique is effective. Although a government's intentions may be crystal clear, the disobedient may correctly calculate that its humiliation in seeking to legitimize its whims would often be a deterrent. British universities now seem bent on borrowing from the same book of civil disobedience. Their response to the British government's assumption that they will help administer a scheme for awarding loans to students to cover part of their living costs has been classic Gandhi: on behalf of almost all British universities, the Committee of Vice-Chancellors and Principals (CVCP) has said that they will do as asked only if there is legislation to compel them.

It is a tortured tale. It is two years since the government set out to limit the direct cost of maintaining British students at British universities at roughly what it is at present. The simple solution would be to peg maintenance grants at their present value (£2,845 in London, £2,265 elsewhere, for the children of not-so-well-off families), but that would be unjust. So there will be a system of loans to supplement grants as they are eroded by inflation. The government already has the legislative authority to proceed. Its first conspicuous mistake was to announce that the commercial banks would administer the scheme without first having asked them.

After a year's uncertainty, the banks last December opted out from the invidious role of being the government's debt-collector among their own potentially most valuable customers. Now the government's casual assumption that the universities would do the job instead is also likely to be frustrated.

Issues

Mr John MacGregor, Secretary of State for Education and Science, says he will call the universities' bluff by arranging for the necessary legal authority, but that will not be simple. Although a suitable amendment to last year's Education Act could be drafted in ten minutes and no more than a dozen lines of text, carrying it through the British Parliament will be a major undertaking, raising again the whole question of whether the British government really understands what higher education is for.

To judge from appearances, it does not. The issue of

student loans is by no means the most serious of the indignities heaped on British universities in the past ten years, but it stems like others from the government's perverse preoccupation with the costs of the universities rather than their benefits. For this sector of the British public education system, policy seems to have flowed exclusively from the principle that costs must not increase. In this case, the government seems prepared to insist on the principle that higher education should not be subsidized in the face of mounting evidence that one of Britain's overwhelming problems in the decades ahead will be a scarcity of skilled people. One might have thought that a government as committed to market economics as the present British government might have worked out that grants should be increased at a time like this, not replaced by loans that are bound to seem more onerous.

Oddly enough, there are many even among the government's enemies who are sympathetic to the notion that a better system than the present should be found for persuading students to universities and for keeping them there. The present system of grants is poorly targeted at those who most need help. The objection to the loans scheme proposed is that it is ill thought-out, and that it will be impossible to administer fairly. The people best placed to devise a workable scheme — the universities themselves — have never been asked, but have only been told what they should do. It is no wonder that they have now rebelled.

What the government should now attempt is what it should have done at the beginning — to find a way of marrying its interest in capping what it spends on student support with universities' now much-sharpened interest in the idea that they should have sufficient students to teach. Yet as things are, hardly any British university is in a position to offer students it would especially welcome onto its books financial support of any kind. They need funds of the kind the government spends as statutory obligations to students, even if in smaller amounts. In return for help of that kind, the universities might well be persuaded to administer a loans scheme of some kind — but it would be better to start afresh, and find one that will work.

Is it too late for the government to swallow its pride and acknowledge that it must find a better way of doing an important job? □



Towards reunification of German universities

- Potential brain drain alarms East
- Deutschmark the irresistible lure

Munich

FEARS that huge numbers of East German students will apply to already overcrowded West German universities drove government ministers from the two countries to meet last week in search of ways to stem the flow. But there will be no quick solutions until the structure of universities in East Germany, and the application procedure in the West, have been fundamentally changed. West German Education Minister Jürgen Möllemann (Free Democrat) and East German Research Minister Peter-Klaus Budig agreed after their meeting in Berlin that the East German universities must be "opened" to many more students if a serious 'brain-drain' is to be prevented.

But Budig, an interim minister in place until East German elections on 18 March, promised no concrete measures to open the universities. University admissions in East Germany are at present bound by strict quotas in all fields. The state provides a free education, along with housing, to all students. The East German government has indicated that it cannot afford more stipends or housing for students. And Möllemann could make no promises concerning the exact numbers or fields of the "numerous" West German professors he said were interested in teaching for a semester or two in the East.

The West German parliament has approved an extra DM50 million (\$30 million) for the Education Ministry in 1990 to help shore up the East German universities through cooperative programmes with West German universities. But the money may not be used for East German universities directly, said ministry spokeswoman Andrea Reidt. The parliament also approved DM600 million in extra help in 1990 for West Berlin, but only DM5 million of that will go to universities. The *Länder* (states), the main providers of university budgets in the West, have yet to offer any help to East Germany in this area.

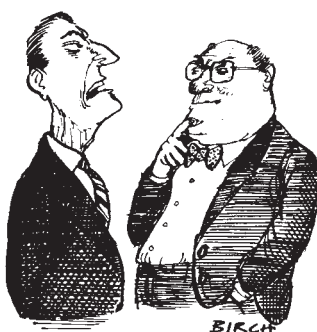
The tens of thousands of East Germans who might soon apply to West German universities represent not only a loss of talent for the east but a burden that could push the West German university system close to collapse. West German universities are already catering for 1.5 million students in a system designed for 800,000. Under current regulations, West Germany allows anyone with a German passport to study if they present the proper credentials, primarily a completed *Abitur*,

or high-school diploma. At individual universities there are admission quotas in a few popular fields such as biology, law or art history. But ironically, East German applicants often stand a better chance than West Germans at being admitted in these fields, said Traugott Klose, an administrator at the Free University of West Berlin. The East Germans often have better grades, and they have been waiting for years to gain admission to university.

West Germany also gives money to students who cannot finance their own studies. These grants, known by their German acronym 'Bafög', would be given automatically to East Germans under the current rules. This would lead to absurdities in Berlin, where students living in East Berlin could commute to university and get rich on their monthly stipend of DM900 — a windfall if converted on the open market into East German marks.

Besides the obvious lure of making a new start in the affluent West, many East German students would like to study fields that were closed to them by the former Communist government. Although 22,000 students begin university each year in East Germany (out of a total of some 27,000 who receive *Abitur*), many were prevented from entering law,

What we really need is
some sort of a wall....



medicine or other desirable fields, said Klose. Some were not allowed to study at all, for political or other reasons.

But Klose considers the popular estimate of "tens of thousands" of East German applicants to be "a gross exaggeration". Budig promised "renewal from the ground up" in the East German education system but Klose was sceptical. "Such a closed system cannot be changed overnight", he said.

Steven Dickman

GENE THERAPY

Clinical trials next step

Washington

Two researchers at the US National Institutes of Health (NIH) are seeking permission from federal review committees to perform a human gene therapy study. If authorized, it will be the first federally approved experiment that attempts to treat hereditary disease by replacing defective human genes. W. French Anderson of the National Heart Lung and Blood Institute and R. Michael Blaese of the National Cancer Institute (NCI) plan to treat children who have severe combined immunodeficiency (SCID) — a rare disease that affects only a few dozen children in the United States.

Patients with SCID lack a functional gene for the enzyme adenosine deaminase (ADA). The absence of ADA causes a build-up of a toxic chemical that destroys the immune system. The researchers will readminister to patients interleukin-2-stimulated T-lymphocytes that have been genetically repaired and carry a correct version of the ADA gene. It is not yet known whether the therapy will have a temporary or lasting benefit.

Before proceeding with clinical trials, Anderson and Blaese must overcome a series of regulatory hurdles. The proposal will first be considered by the human gene therapy subcommittee of the NIH Recombinant DNA Advisory Committee, or RAC, on 30 March, and then by the full RAC on 1 June. Nelson A. Wivel, director of the Office of Recombinant DNA Activities at NIH, says that is "conceivable that the subcommittee [for gene therapy] may want more data" and that they "may not have finished their deliberations by the end of the meeting". The protocol must also be approved by the Food and Drug Administration.

In a separate proposal, the human gene therapy subcommittee will also consider a proposal from Anderson, Blaese and Steven A. Rosenberg of the National Cancer Institute to remove the limitation of patient numbers on the original human gene transfer experiment, which was approved in January 1989 amid much controversy (see *Nature* 337, 294; 1989).

This protocol, which forms the basis for the proposed human gene therapy experiment, has so far involved six patients. Anderson and colleagues are authorized to treat up to ten cancer patients with tumour-infiltrating lymphocytes (TIL) marked with a gene for neomycin resistance. Although the gene confers no therapeutic benefit to the patient, it allows researchers to determine whether the cancer-killing TIL cells 'home' to the tumour site.

Diane Gershon

JOURNALS

Errant survey draws fire

Washington

A BITTER dispute between two US science publishers took a bizarre turn this month when it was revealed that one had been surveying its customers under the letterhead of an unrelated foundation.

Last month Gordon and Breach Science Publishers, Inc. sent surveys to university libraries on the stationery of the London-based Foundation for International Cooperation — apparently without the foundation's prior approval. Among the 19 question included in the surveys were several that asked if the libraries had cancelled subscriptions as a result of an article in the July 1988 *Physics Today* that ranked physics journals by a ratio of their cost and impact.

The original *Physics Today* article put Gordon and Breach at the bottom of a list of 24 publishers. Charging "unfair comparative ranking", Gordon and Breach then sued the American Institute of Physics (AIP) and the American Physical Society — the organizations that publish *Physics Today* and the *Bulletin of the American Physical Society* — as well as the article's author, Henry Barschall, a retired professor of physics at the University of Wisconsin in Madison (see *Nature* **341**, 28 September 1989).

Despite the questionable circumstances surrounding the distribution of the library survey, Frank Forrest, a spokesman for the company, says results from it could potentially be used as evidence in court to prove that Gordon and Breach was indeed damaged by the article in *Physics Today*.

The foundation disclaimed any knowledge of the survey when librarians brought it to the attention of the Association of Research Libraries and the Chroni-

cle of Higher Education earlier this month. Gordon and Breach sales director Christopher Schneider says the use of the letterhead and the signature of Maurice Levy, the foundation's director, without permission was due to secretarial error. He says that Martin Gordon, chairman of the publishing company, is on the foundation's board and apparently gave the go-ahead before checking with officials. "The survey may have gone out a little prematurely," Schneider says.

Despite the imbroglio, Forrest says that an agreement has subsequently been reached with the foundation to begin a project — of which the survey will be part — on the methodology of price-comparison studies and the importance of journal prices in purchasing decisions. The study will be for "market research purposes".

The publishing firm has already received dozens of survey responses from libraries, but no results have been released.

Four lawsuits against *Physics Today's* publishers have been filed by Gordon and Breach in three countries — France, West Germany and Switzerland — that have laws against "comparative advertising" (the United States does not). A Swiss court has thrown out one claim for being filed in the wrong court, although the others are still pending. AIP lawyer Richard Meserve says the suit appears to be "nuisance" litigation, intended to discourage comparative rankings such as the *Physics Today* article. He points out that even if Gordon and Breach won its suits, it would only be entitled to damages in those countries in which they have been filed, which would account for a very small proportion of Gordon and Breach sales.

G. Christopher Anderson

MIT PRESIDENCY

Thanks . . . but no thanks

Boston

PHILLIP A. Sharp, whose recent nomination to serve as president of Massachusetts Institute of Technology (MIT) was greeted with almost unanimous approval, turned down the job last week, surprising the MIT community and leaving an embarrassing vacuum in the selection process. Sharp was the only candidate proposed by the university's search committee, and his selection was all but assured (see *Nature* **343**, 681; 22 February 1990).

In a statement, Sharp apologized for his "indecisiveness" and said that he was "challenged and honoured" by the offer of the MIT presidency. But he said he could not bear to give up his research and teaching. "I could not fill that void in my life with anything else," he said.

Although Sharp was the MIT search

committee's only nominee, his decision makes him the third candidate to have removed himself from consideration. David Baltimore, widely considered a front runner, accepted the presidency of Rockefeller University, effectively declining a potential offer from MIT. John Deutch, currently MIT's provost, also decided not to try for the post after objections to his possible selection were raised by students and faculty.

Henry Jacoby, chairman of MIT's faculty, expressed his disappointment at Sharp's refusal in an official letter to the faculty, but went on to say that Sharp was "one of many highly qualified candidates for the presidency". While that may be true, few are hazarding any guesses as to who will finally take the post.

Seth Shulman

ANIMAL RIGHTS

UK researchers to establish defence fund

London

BRITISH scientists involved in experiments on animals may soon be able to receive help with legal fees to fight libel actions against animal rights groups or the press. The Research Defence Society (RDS) has set up a legal defence fund, which its director, Mark Matfield, hopes will "deter antivivisection activists from mounting campaigns against individual medical researchers". Matfield believes the fund will reach at least £100,000, and expects most contributions to come from scientific and learned societies.

The fund has been welcomed by scientists involved in work with animals. Nick Wright, associate director of clinical science with the Imperial Cancer Research Fund (ICRF) expects the defence fund to be called upon "quite often" and says it will be particularly valuable to researchers attacked in "protracted press campaigns". Wright says that the ICRF would pay legal fees if one of its researchers was libelled, but many scientists do not have this protection.

Professor Colin Blakemore of the University of Oxford, himself the victim of vilification in the national press, thinks the defence fund will act as a deterrent against libel. But he says that the fund is only the first step in the fight against the escalating attacks on researchers by animal rights groups.

Blakemore believes that recent press campaigns against animal researchers might have been curtailed if "appropriate organizations" (research councils, medical charities and scientific societies) had stepped forward to explain the value of research involving animals.

Peter Aldhous

AGRICULTURAL RESEARCH

Dismay at AFRC insect unit

London

RESTRUCTURING at the Agricultural and Food Research Council's (AFRC) Unit of Insect Neurophysiology and Pharmacology at the University of Cambridge will lead to 8 redundancies later this year. A review of the unit's activities was expected, following the death of its director, John Treherne, last year, but some researchers in the field are surprised at the dismissal of scientists with an international reputation. One of the sacked researchers, Simon Madrell, is a Fellow of the Royal Society, widely regarded as a productive scientist with an excellent publication record.

The redundancies are all the more surprising, given that the unit is to focus its future research on the molecular basis of interactions between cells in insects. Both Madrell, and another of the dismissed scientists, Helen Skaer, have worked in this area.

Peter Aldhous

Gloom in Latin America

São Paulo

LATIN America has 8.3 per cent of the world's population, but only 2.5 per cent of its engineers and scientists, 1.8 per cent of the resources given to research and development and 1.3 per cent of the authorships of scientific papers. These discouraging statistics provided the backdrop for last week's first meeting of heads of science and technology agencies in Latin America, held in São Paulo at the 'Latin America Memorial', a cultural centre created to help speed integration among the peoples of the continent.

One suggestion was that governments should consider "debt for science" arrangements with foreign creditors. The idea was put forward by Crodowaldo Pavan, president of Brazil's National Council for Scientific and Technological Development (CNPq). Both Pavan and Israel Monsewer, head of Uruguay's National Council for Scientific and Technical Research, said the 80s were "a lost decade" for Latin America's development.

Despite calls for regional integration, there is a widespread lack of cooperation in research. Bolivia's Jose Antonio Zelaya, responsible for Science and

Technology development at the country's Ministry for Planning, said that he would like to see more multilateral work in areas that affect more than one country, like Amazon research or the study of the River Plate basin. But, participants agreed that cooperation only works well when it is based on specific projects, not "letters of intent" signed by governments.

The meeting was not without dire warnings and prophecies. Some participants fear that interest in Eastern Europe will hit investment and debt-relief in Latin America. "We may lose the last train to the coast", said Brazilian diplomat Celso Luiz Nunes Amorim, who heads the Cultural Department at the Ministry for Foreign relations. "Integration" could help, he said. But another Brazilian, Geraldo Lesbat Cavagnard Filho, director of the Nucleus of Strategic Studies at the State University of Campinas disagreed. Only integration with the rest of the world will provide long-term benefit, he said. Uruguay's Monsewer reminded everyone that Latin America's participation in the world export market dropped from 7.7 per cent in 1960 to 3.9 per cent last year.

Ricardo Bonalume

AMAZONIA

Red tape still a problem

São Paulo

A NEW decree regulating the conduct of scientific expeditions in Brazil came under fire last week at a meeting of the Brazilian Society for the Progress of Science (SBPC). "The intention of the decree is not to foster international scientific cooperation" said SBPC. The society asked government to revoke the decree.

The new decree, passed last month, replaced a 1969 decree which has been a constant source of complaint both inside and outside Brazil, on the grounds that it makes it difficult for foreign scientists to visit the Amazon and other regions of Brazil without years of paper work. "The new decree is better than the old, but it still doesn't settle the basic problem, which is to provide incentives for international cooperation," says anthropologist Eunice Durham, one of SBPC's vice-presidents.

The Ministry of Science and Technology (MCT) defends the new decree. The ministry's secretary-general, mathematician Lindolpho Carvalho Dias, says that if the decree has its faults, scientists need to present suggestions for amendments and not ask to abrogate it.

Expeditions are a sensitive topic in Brazil, and the decree actually avoids using the word. "Expedition" reeks of colonialism," says Carvalho Dias. The

decree talks instead of "collection, by foreigners, of scientific data and material in Brazil".

Carvalho Dias insists that the goal of the decree is to emphasize cooperation. The old decree said that every expedition had to have at least one Brazilian scientist in attendance, which turned the Brazilian researcher into a sort of "police" responsible for the visitors. The new legislation calls for participation of a Brazilian institution instead. This means that the documents required for a permission to "collect" data and material need not be sent to a Brazilian consulate or embassy abroad, but are the responsibility of the partner institution in Brazil.

Waiting periods are also reduced. Under the old decree, foreigners had to submit all documents six months in advance and then wait for permission to be granted. This could take more than a year. The new decree says that it is the ministry who has to give an answer with a explicit deadline, set at four months — except under "exceptional" circumstances.

The biggest hurdle, according to SBPC, is the need to consult several different government agencies, depending on the goal of the expedition. To visit the northern region inhabited by the Yanomami people, for example, the list would be very long.

Museums make news

LONDON'S museum world, not a haven of peace in recent years, has been in the news again. The Natural History Museum has been the target of protests (page 9), and across the other side of the City, a new museum has been jeopardized by the loss of its major source of funding. The Museum in Docklands project, launched in 1982, may have to cease work on 31 March with the withdrawal of support from the London Docklands Development Corporation (LDDC), the body charged with overseeing the development of the large area vacated by London's docks when the coming of larger ships and containerization made them redundant. The current slump in property values in southeast England



had led the LDDC to look for money-saving measures, although supporters of the museum project argue that without such amenities the docklands area will remain unattractive to developers and would-be inhabitants.

Among the 100,000 exhibits in store and awaiting a home are a number of scientific instruments made in the area, including the 'octant' shown above, made in 1770 by Thomas Ripley of Hermitage Basin.

C.W.

Because the region is close to the border and there are conflicts with illegal gold prospectors, the military would have to be consulted. Because of the presence of native people, the federal agency dealing with them would need to grant permission. And if geologists are involved the agency responsible for mining will have to have its say. If the research strays into a natural preserve, the environment body will also be involved. The Ministry of Foreign Relations, will also want to make its views known, on top of the agency the decree says is "responsible" for granting permission, the National Council for Scientific and Technological Development (CNPq).

Researchers at the National Institute for Amazon Research complain that government red tape is already luring foreigners to study the rain forest in Costa Rica or Peru, instead of coming to Brazil. Scientists would like CNPq to have sole and complete responsibility for expeditions.

Ricardo Bonalume

Concern in committee

London

AN all-party House of Commons committee has announced that it is "very concerned over serious financial difficulties facing many universities". The statement came in the Public Accounts Committee's report on financial problems at universities, which was prompted by the financial crisis at University College, Cardiff, during the 1980s.

Cardiff's problems arose from the failure to reduce staff numbers in the face of a smaller grant allocation in the early 1980s. Despite pressure from the Department of Education and Science (DES) and the old University Grants Committee (UGC), the college's deficit worsened until the DES withheld its grant in February 1987. This was restored a month later, but only after a new team of financial managers had been appointed and Bill Bevan, the college's principal, had stepped down. University College, Cardiff, has now been merged with the University of Wales Institute of Science and Technology.

As expected, the committee's report condemns the college's financial management up to 1987. "There was no credible and co-ordinated financial and academic planning", the report concludes.

The report also vindicates the DES's "unprecedented action" of overriding university autonomy to "restore financial discipline" to the college. But the UGC is criticized for failing to take decisive action before the college's problems had become deeply entrenched, and for not reporting the situation to parliament earlier.

The establishment of the Universities

Funding Council (UFC, which replaced the UGC in April 1989), is welcomed by the Public Accounts Committee, which expects the UFC to take a more interventionist role than the UGC to ensure economy and public accountability of university funding. The UFC is currently monitoring the final stages of universities' restructuring programmes, which have involved staff cuts, mostly through early retirement or voluntary redundancy. Sir Peter Swinnerton-Dyer, chief executive of the UFC, was due to appear before the Public Accounts Committee this week to give evidence on the accidental misuse of some funds intended for restructuring by some universities.

Despite restructuring, many universities still face financial problems. The UGC reported in 1987 that universities' costs were expected to rise faster than their incomes. The University of London, which predicts an accumulated deficit of £46 million by 1992-93, on an annual budget of over £600 million, is the UFC's major concern. Of London's 15 major colleges, only the London School of Economics is not in debt. Lord Flowers, London's vice-chancellor, has discussed the problem with the UFC, which will debate the issue at its next meeting in March. The UFC stresses that it is not accusing London of financial mismanagement; the university's problems stem mainly from the costs of operating in an expensive capital city. London's management believes the university is underfunded to the tune of about £10 million per year, but the UFC has not yet accepted this figure.

Peter Aldhous

MEDICAL RESEARCH

Imperial's Wellcome support

London

THE latest grant round from the Wellcome Trust, a UK charitable foundation, includes £4 million for Imperial College, London, to set up a new research centre in parasitic diseases. The grant is one of the largest single awards ever made by the trust.

The centre will be based around a core of scientists from Imperial's biology and biochemistry departments, directed by Professor Roy Anderson, an epidemiologist. Anderson expects the centre to employ 21 new staff at Imperial, and four researchers working in Africa, Asia and South America. The emphasis on close links with countries most heavily afflicted with parasites, he says, will be an important part of the centre's effort to combat the diseases.

Wellcome has also agreed to provide funds for the European arm of the Human

Genome Organization (HUGO) over the next three years. Michael Morgan, molecular and cell science programme director for Wellcome, says that a sum "in the order of £200,000" will be used in the coming year to set up a London office with two administrative staff, and to pay for European-based HUGO members to attend HUGO council meetings.

HUGO was launched in 1988 to help coordinate international efforts to sequence the human genome, but has been attacked for its apparent inactivity and failure to attract significant funding (see *Nature* 342, 724; 1989). Bronwen Loder, who has been running an "embryonic" HUGO office in London, with limited financial support from the Imperial Cancer Research Fund (whose research director, Sir Walter Bodmer, is HUGO president), describes the Trust's grant as "immensely welcome".

Peter Aldhous

BSE compensation

London

IN a policy reversal, the UK agriculture minister John Gummer announced last week that British farmers will receive full compensation for the destruction of cows infected with bovine spongiform encephalopathy (BSE), rather than half the market price. The increase should remove any remaining temptation for farmers to sell infected cows for human consumption.

Veterinary and health groups have been campaigning for full compensation, but the Ministry of Agriculture, Fisheries and Food says that the decision is not the result of lobbying, or of concern for public health. A ministry spokesman said that the change reflected government recognition of farmers' loss of a valuable export market (the European Commission decided recently to ban the export from the United Kingdom to other Community countries of all live cattle older than six months), and the increasing number of herds in which several BSE cases were being found. P.A.

Lasker prize hiatus

Washington

IN a surprise announcement, the Albert and Mary Lasker Foundation said last week that it would suspend its respected medical research awards for the year to rethink their future.

"We are reviewing our whole programme", says Alice Fordyce, executive vice-president of the foundation and director of the medical awards. She stressed that the move was not based on financial considerations, but was rather because, after 44 years, it had become necessary to reappraise the impact of the award. "We need to get feedback from the community. . . . Were we making a difference? Should we be doing things some other way?" she asks.

The hiatus is the first since 1961 when the awards were put off for one year to accommodate bureaucratic changes. Although lack of money is said not to be a factor, the foundation's assets are down from \$4.5 million in 1980 to \$2.4 million.

Each winner normally receives a \$15,000 honorarium at a ceremony held each September. Since the awards were established in 1944, 46 winners have gone on to receive Nobel Prizes.

G.C.A.

ESA names new DG

London

JEAN-MARIE Luton, director general of the French National Centre for Space Studies (CNES), will replace Reimar Lüst as director general of the European Space Agency (ESA) for four years from 1 October this year. Luton has a background in geophysics research, and is currently a French delegate to the council of ESA. From 1984 to 1987, he chaired ESA's administrative and finance committee.

P.A.

Cautious start for AAAS

Washington

THE American Association for the Advancement of Science (AAAS), publisher of the journal *Science*, is to launch a new all-electronic rapid-publication journal tailored principally to the 'data-intensive' areas of science where conventional journals have difficulties in providing sufficient space.

Plans for the new journal, a joint venture between the AAAS and the Online Computer Library Center (OCLC) of Dublin, Ohio, were made public last week in a surprise announcement at the annual meeting of the AAAS in New Orleans. The new journal represents the first attempt by a major US scientific organization to offer electronic publication and may set a trend for the future. But *Science* is not about to vanish — the new journal will be a completely separate publication, even though planned in the same office.

Daniel E. Koshland Jr, editor of *Science*, says that a key advantage of the new journal will be in the amount of data that it can publish. "A lot of very good scientists do not send articles to *Nature* or *Science* because there is no way they can get enough data to be convincing in the size that we allow," says Koshland. The new journal will offer a new "intermediate" niche, part way between a conventional journal and a database.

The new journal will be referred in the same way as *Science* and "will require an editorial board," Koshland says, "that can decide, 'Yes that paper is at the right intermediate level of information'".

Papers that involve crystallographic coordinates and large amounts of sequence data (where both *Science* and *Nature* will now often publish only the 'bits judged interesting', says Koshland) are obvious candidates for publication in electronic form, but they are not the only areas that the AAAS is thinking of.

Richard S. Nicholson, Executive Officer of AAAS and the man behind the deal with OCLC, says the new journal will be attractive in any area where readers want to have access to the data behind a manuscript and manipulate it for themselves.

Another appealing feature of the new journal is that a paper would be published — in the sense that it would be added to the journal's electronic archive — the instant that it had passed peer review. There will be no need for it to join a queue for space in an upcoming issue of the journal.

Access to the journal will be possible through electronic networks, but Koshland stresses that the availability of data in electronic form is the main attraction. He expects that many 'papers' will be submitted and sent out to referees and subscribers on disc.

For OCLC, AAAS's partner in the new journal, the joint venture is attractive because it will one day help libraries overcome storage and administration problems — electronic journals never need be sent away to the binders. OCLC is a non-profit computer library service and research organization, which was founded in 1967 by the university presidents of the state of Ohio. Since then it has grown into a network serving all 50 US states and 39 countries, offering computer services and access to the world's largest bibliographic database (containing 331 million location listings) to over 10,000 libraries. In 1988 it dealt with an average of 2.6 million transactions a day, arranged over 4 million interlibrary loans, and took in revenues close to \$100 million.

According to OCLC spokesman Philip Schieber, the organization would like to move towards providing electronically not just standard bibliographic data (authors, titles, etcetera) but also data necessary for subject searches (a system is in experimental use) and eventually the full text itself. Over half of all library acquisitions are serials, he says, and libraries are keen to find innovative ways to deal with the growing costs of storing and subscribing to journals. Early involvement in an electronic journal is a logical step for OCLC in a competitive field in which "everybody is still just putting their toes in the water", says Schieber.

Nicholson is confident that publication of the journal can begin next year although many details have still to be worked out. Will the journal be able to accept advertising? Neither side in the joint venture is prepared to say. Advertising is possible — some electronic newsletters force subscribers to give up several lines on their screens to a changing series of commercials. And how will subscribers pay for the journal? Will there be a fixed subscription or will users pay according to how much they wish to see? Nicholson says that many possibilities are under consideration.

Elsewhere, schemes with similar objectives or using similar methods are at a similar stage of development. In Europe, for example, a consortium of Elsevier, Springer, Pergamon and Blackwells, all major journals publishers, are believed to be on the point of selling packages of information based on the contents of existing journals, but in electronic form.

Nature, with which *Science* is often compared, has for some months suggested to the authors of papers accompanied by large amounts of data that this should be circulated separately as "supplementary information", with the intention that this should eventually be available electronically.

Alun Anderson

'KGB hackers' get off lightly

Celle, West Germany

THREE West Germans found guilty of breaking into US federal computers and selling information to the KGB, the Soviet intelligence agency, received relatively mild punishment last month from a West German court because of the incompetence of their attempt at espionage. The men were given sentences of fourteen months to two years but were released on probation for three years. They were also required to pay a fine of DM18,000, a fraction of the DM90,000 (\$54,000) they received from the KGB. The prosecutor, Max-Ekkehard Kohlhaas of the Federal Prosecutor's Office, had requested a longer jail term for one of the defendants, Peter Carl, because he had been the most important link to the KGB.

The sentence was light, the court explained, because little damage had been done. "What the defendants offered did not interest the Russians", said the chief judge, Leopold Spiller, "and the defendants did not gain access to anything very interesting." Two of the sentenced hackers, along with a third, Karl Koch, who died in mysterious circumstances in May 1989, had offered to sell the KGB everything they knew for DM1 million, but the KGB turned them down.

The attempted spying was unearthed by US astronomer Clifford Stoll, who discovered in 1986 that hackers had broken into the computer at Lawrence Berkeley Laboratories (LBL) in California, and were trying to gain access from there to hundreds of computer systems at military bases and research institutes (see *Nature* 333, 105; 1988). The hackers admitted to having had dealings with the KGB but denied that they had been tracked by Stoll. They apparently convinced the court of that, because Stoll admitted that other hackers had also been able to enter the LBL computer, and what might have been a test case for West German legislation against hacking therefore became an open-and-shut case on the more serious, but easier to prosecute charge of espionage.

Last week, the cabinet in Bonn created a new central authority to handle computer security. The Federal Office of Computer Security, which is attached to the Interior Ministry, will serve as a clearing house for data about computer misuse in industry and government. In the past, West Germany had only an office for protecting military computer security. The new office will help the computer industry and user community to develop strategies against misuse. It will also keep a closer watch on the West German telephone system, which was identified as a weak link in the US break-ins.

Steven Dickman

MISCONDUCT IN SCIENCE

Australia's new guidelines

Sydney

THE Australian Vice-Chancellors Committee (AVCC) has released guidelines aimed at helping universities deal with academic fraud "to ensure that the integrity of Australian university research was not damaged by the few cases of academic fraud highlighted recently in Australia, the United States and the United Kingdom." The AVCC guidelines define the types of academic fraud and the procedures for dealing with allegations of fraud; defined to include fabrication of data, plagiarism and the publication of misleading statements regarding authorship. The guidelines recommend that all authors must sign a form confirming their involvement in a paper before publication.

The report, written by Professor David Caro, former Vice-Chancellor of the University of Melbourne, puts the onus squarely on supervisors to ensure the validity of students' work and places restrictions on the number of students one person can supervise. If allegations of fraud or misconduct are made, the AVCC recommends that the staff member concerned be informed in writing and given details of the complaint. The staff member has thirty days to respond to the Vice-Chancellor.

The new guidelines change the circumstances under which an inquiry terminates. Under present university employment

conditions, an investigation must end if the person under investigation resigns. In 1987, for example, investigation of allegations that Professor Michael Briggs, Dean of the Medical School at Deakin University in Melbourne, fabricated data on oral contraception were halted when Briggs took early retirement and moved to Spain. Under the AVCC recommendations, if the accused investigator resigns, the university must convene an inquiry to report on the status of the research in question and on any remedial action needed to protect students, academic colleagues and the public. This includes contacting journals in which allegedly fraudulent papers have been or are about to be published and funding bodies which have contributed to the research.

The Caro report stresses "absolute confidentiality and reasonable speed in the early stages of an investigation to protect both the complainant and accused" but leaves decisions on partial disclosures in individual cases to vice-chancellors, who are also responsible for authorizing preliminary investigations and determining the form of the investigation. The guidelines, according to Caro, are a preventive measure rather than a response to escalating levels of fraud in Australian universities. "There have only been three or four examples of fraud in this country".

Tania Ewing

INTERNATIONAL COOPERATION

Japan still an island academically

Washington

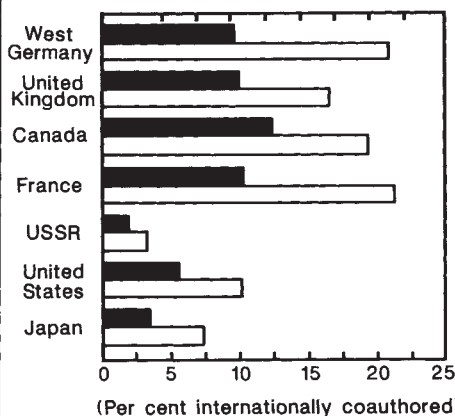
DESPITE its scientific achievements, Japan appears still not fully integrated into the world academic community when new data on who Japanese work with, and who they cite in their papers, are considered.

Statistics charting the gradual internationalization of research are reported in the new issue of the US National Science Board's biennial report, *Science and Engineering Indicators — 1989*. As shown above, all the major western science nations have come close to doubling the percentage of their papers that are co-authored with foreign scientists over the last decade. But Japan has a far lower rate of foreign co-authorship than any other western nation.

Statistics on who Japanese cite in their papers come from an Institute for Scientific Information (ISI) survey published in the latest issue of the journal *Science Watch* (February 1990).

ISI analysed 2.92 million citations made between 1984 and 1988 to some 897,000 papers published in 1984. After correcting for differences in the total numbers of papers from each country, ISI found that

the scientists of every nation tend to refer to work from fellow countrymen more often than to work from people elsewhere. But the preference for self-citation is most pronounced in Japan. Citation links between Japan and North America or the



Internationally co-authored articles by country: 1976 and 1986.

European Community are only half as strong as the links between North America and the European Community.

Alun Anderson

ROCKET LAUNCHERS

New setback for Ariane

Paris

EUROPE's commercial satellite launch consortium, Arianespace, suffered a setback last Friday when one of its new generation Ariane-4 launchers exploded shortly after lift-off. The payload of two Japanese telecommunications satellites was destroyed. This was the fifth failure in the thirty-six missions since the first Ariane launch in September 1979.

According to a communique issued from the Arianespace headquarters outside Paris, a sudden drop in pressure occurred in the combustion chamber of 1 of the 4 strap-on boosters of the first-stage propulsion system, just over 6 seconds after lift-off. Automatic compensation by the other three boosters, to correct for the loss of thrust, caused "excessive loads" on the launcher, leading to the explosion at about 9 km altitude, 12.5 km from the launch pad in Kourou, French Guyana.

A committee of enquiry is being set up, with representatives from the different aerospace manufacturers involved in Arianespace together with representatives of the European Space Agency. The composition of this committee should be decided by Tuesday 27 February. In the meantime, "detailed technical analysis of the failure" has been started, and future launches are to be suspended until "a complete understanding of the accident has been reached and corrective actions defined and implemented".

One of the satellites on board, BS-2x, was meant to back up and eventually replace the BS-2b satellite which is used for direct broadcasting to the entire Japanese archipelago. The satellite was also to be used for experimental trials of Japanese high-definition television broadcasting technology, pending the launch later this year of BS-3 by a Japanese H1 rocket. The other satellite was Superbird B, a telecommunications satellite built for the Japanese Space Communications Corporation.

Having won over 50 per cent of world commercial launches while the US competition was out of the race following the shuttle disaster, Arianespace is now facing a tougher time in the 1990s. Not only is the US competition back, with General Dynamics' Atlas Centaur launcher, but the Soviet Union, China and Japan also want a share of the limited market.

Nevertheless, Arianespace still has an order book of 32 satellites, worth about \$2,200 million, awaiting launch and hopes to make up for the lost time within twelve months — provided of course that the problem is not as knotty as the one affecting the third-stage motor in May 1986 that stopped all Ariane launches for 14 months.

Peter Coles

International interest

Kobe

At an international symposium on synchrotrons held in Kobe two weeks ago, scientists from Europe, the Soviet Union and the United States described a variety of next-generation synchrotrons whose purpose is not to accelerate particles but to produce intense beams of radiation ranging from ultraviolet to X-rays.

Synchrotron light sources are finding a wide range of applications in experimental physics and technology, and it is Japan that seems likely to have the most powerful machines by the end of this decade.

The first of the new generation machines to come on line will be the European Synchrotron Radiation Facility (ESRF), now in its third year of construction at Grenoble, France. Ten beam lines from the 6-GeV ESRF are expected to be available in mid-1994, with expansion to 30 lines by 1998, according to Rupprecht Haensel of ESRF. Projects including materials science, protein crystallography (2 lines) and surface diffraction studies have already been selected for the first eight beam lines.

In the United States, the 7-GeV Advanced Photon Source (APS) at Argonne, Illinois, is not expected to be completed until 1995 but the facility already has a mailing list of about 3,500 potential users, according to G.K. Shenoy of APS. One crucial technical hurdle that APS must overcome is the development of well-cooled optics that can handle the tremendous beam power that will be focused on samples. The APS engineers intend to solve this problem by using liquid gallium rather than water as a coolant, and their experience will aid Japan's plans to build the world's most powerful synchrotron, the 8-GeV Super Photon Ring (SPRING-8) facility at Harima Science Park City west of Osaka, construction of which begins this month (see *Nature*, **343**, 582; 15 February 1990). SPRING-8, which is expected to undergo its first tests in 1997, may be capable of achieving about ten times the beam brilliance of APS, according to calculations presented by the SPRING-8 design team.

With such high brilliance, it may be possible to take picosecond snapshots of the reaction kinetics of proteins. And this could open up a "completely new era in crystallography and structural chemistry", according to Janos Hajdu of the University of Oxford in the United Kingdom, who has carried out pioneering work on enzymes and viruses at the Daresbury synchrotron.

But Hajdu warns that there is a need to put more effort into the development of detectors that operate at such speeds if the new synchrotrons are to reach their full potential. Japan has made a contribution

on this front with Fuji Film's development of an imaging plate that may be able to take high-quality snapshots at the picosecond level. But there is a need for real-time detectors, Hajdu says.

Soviet scientists have an ambitious plan to build a 10-GeV synchrotron, Siberia-3, perhaps at Dubna near Moscow, G. Kulipanov says. But "we have no money" and "500 million roubles is not so easily obtained at the moment".

Physicists at the High-Energy Physics Laboratory (KEK) in Tsukuba have long

HIGH-ENERGY PHYSICS

Big linear collider proposed

Tokyo

HIGH-ENERGY physicists in Japan are hoping to build a huge linear collider that will compete for results with the United States' Superconducting Super Collider (SSC). But, at the earliest, their proposed collider could not be built before the beginning of the next decade, and in the meantime many Japanese physicists are keen to participate in experiments at the SSC. Whether the Japanese government, however, has a similar enthusiasm to help build the SSC remains far from clear.

The Japanese Linear Collider (JLC), which is being promoted by scientists at the High-Energy Physics Laboratory (KEK) in Tsukuba, would cause positrons and electrons to collide at an energy of 1 TeV. This is less than the combined 40 TeV proton-proton collisions of the SSC, but the lower energy is partly made up for by the 'cleaner' collisions that positrons and electron produce, and the scientific goals of JLC are the same as those of the SSC, according to Seigi Iwata, director of the Physics Department at KEK.

The Ministry of Education, Science and Culture (MESC) has yet to decide whether to go ahead with JLC, but has provided a few hundred thousand dollars for research and development over the past few years. Some funds for JLC have also come from a cooperative US-Japan science and technology agreement between KEK and the Stanford Linear Accelerator Center, California, Iwata says.

The JLC would consist of two linear accelerators, one for electrons and one for positrons, facing each other. With present technology, each 500 GeV linac would have to be about 50 km long, but the developers of JLC plan to keep the total length of the collider down to about 16 km by developing powerful new klystrons to accelerate the particles. Even at this length, Japanese land prices would make JLC's cost prohibitive, an obstacle KEK researchers plan to get around by building the accelerator at a depth of 50 to 100 m.

had a plan to convert the electron-positron collider TRISTAN into a gigantic 10-GeV synchrotron once the collider outlives its useful life in a few years time. Conversion could be carried out at very low cost — the order of ¥10,000 million (\$70 million) or about one tenth the cost of SPRING-8 — and this seems "the biggest possibility" for the final fate of TRISTAN according to Seigi Iwata, director of the Physics Department at KEK.

Several Japanese companies have mini-synchrotrons for the development of VLSI (very-large-scale-integrated) circuits and people from industry were at the symposium.

David Swinbanks

The Ministry of International Trade and Industry (MITI) is currently drafting new legislation that is expected to limit land-ownership rights to a maximum depth of 50 metres. MITI's legislation is intended to promote the development of underground cities and power stations but, according to Iwata, Japan's high-energy physicists are also waiting for "a good new law from MITI".

For this and budgetary reasons Iwata's "best hope" for the start of construction of JLC is 1995. Construction will take "at least five years". Iwata is not prepared to estimate a total cost for JLC at this stage because he says that will depend very much on what is achieved in research and development over the next few years. But JLC will cost more than KEK's electron-positron collider, TRISTAN.

Without a next-generation collider of their own until the next century, many Japanese physicists are very keen to participate in the experimental phase of SSC, Iwata says. But others, particularly those in the universities, would prefer to collaborate with the European Laboratory for Particle Physics (CERN) in the operation of their proposed LHC (Large Hadron Collider), which might be built in the latter half of the 1990s.

Participation in the LHC is expected to cost less than in the SSC. So far, Japan has made no commitment to contribute to the construction costs of the SSC.

Last year, a US mission visited Japan to explain the project to government officials. But since then, there has been no contact, and Japan has made no decision on SSC, according to Yubun Narita, director of scientific affairs at the Ministry of Foreign Affairs. At present, there seems little incentive for Japan to join the construction phase. "If Japanese companies are not allowed to provide superconducting magnets for SSC, what is the point of Japan contributing to construction costs?" asks one leading Japanese physicist.

David Swinbanks

US POLITICS

High-cost of democracy . . .

Washington

YEAR-END figures released this month by the Federal Election Commission (FEC) show that science interest groups are no strangers to the time-honoured tradition of trying to win political influence with hard cash.

The National Education Association (NEA), an organization that supports both secondary and higher education, spent more than \$336,000 on politicians last year, spread out over nearly half the members of Congress. Based on a grading system that ranks each politician's voting record and political platform, the association targets legislators who are likely to support new facilities, funding for research or other potential benefits for university and pre-college education.

Other major science-related organizations that spent from tens to hundreds of thousand dollars on congressional campaigns include big biomedical companies such as Smith Kline Beecham and Monsanto, membership organizations such as the American Medical Association, and even government-funded laboratories such as General Atomics, a fusion research contractor.

And, to no surprise, the top dozen aerospace contractors outspent nearly everyone else. They distributed over \$2 million last year to support a core group of some 150 influential legislators — more than a quarter of Congress.

The FEC figures show that the heads of six of the most important congressional committees that determine federal science funding were given an average of about \$470,000 each, more than twice the congressional average. The source of such largesse is not the companies and associations themselves, but the Political Action Committees (PACs) they create for the express purpose of contributing to congressional campaigns. No single PAC is permitted to give more than \$5,000 per year, so the large totals represent the growing number of special-interest blocs eager to influence legislation.

PACs are one of the most visible creations of the 1970s' campaign finance reforms. Although companies and associations cannot directly give money to candidates, they can set up — and pay the overhead for — PACs to which their employees or members contribute. By allowing individuals to pool their resources, PACs have emerged as the favourite way for corporations and associations to influence politics. Such PACs accounted for nearly \$400 million in campaign contributions last year — up to half the total contributions in some races.

Senators who chair committees receive an average of nearly \$40,000 each from science-related PACs, while their col-

leagues in the House of Representatives averaged almost \$30,000. Total spending on congressional races last year by science and technology-related PACs came to over \$10 million.

For their money, the PACs can legally expect little more than a promise that their views will be considered — and sometimes solicited — about legislation that could affect them. Of course, the voters who make up the constituency of every member of Congress are entitled to the same assurance. But when the time comes to get outside reaction on legislative issues, the names with cheques attached often get the first call. "It can determine whether a call gets returned, a letter gets written, a witness asked to testify. It buys access", says John Deeken, an aide to Senator David Boren (Democrat, Oklahoma).

"You can't buy a politician, but [a contribution] does get your foot in the door", agrees Ivette Torres of the NEA. Critics worry that the increase in PAC spending (since 1979 the number of registered PACs has more than doubled) threatens to shift the balance of US politics away from the voters at large.

"The biggest risk of PACs is that they have evolved into a second set of constituents. There is now a split between the traditional constituents — the voters — and the cash constituents", says Larry Makinson, an analyst with the Center for Responsive Politics, a bipartisan research group. Rather than the democracy envisaged in the constitution, where legislators answer first to the voters in their home state, legislators are now best known — and supported — for the committees they serve on. For example, Senator Bennett Johnston, the powerful head of the energy and water appropriations subcommittee that funds the Department of Energy, received nearly \$1.3 million in PAC contributions last year, almost ten times the congressional average.

But some legislators are beginning to rebel. Senator Boren and Senate majority leader George Mitchell (Democrat, Maine) have introduced a bill that would limit PAC contributions to \$100,000 in the House and to between \$190,950 and \$825,000 in the Senate depending on state size. The aim is to limit the impact of PACs on the political process. Because PACs favour incumbents and legislators on important committees, in 1988 four times as much PAC money went to incumbents as challengers. With more than a quarter of the Senate already co-sponsoring the Boren-Mitchell bill, many analysts believe that the PAC system of campaign finance could be challenged seriously this year and that limits on PAC contributions are likely.

G. Christopher Anderson

HUMAN REMAINS

Dublin victory for aboriginal protest

AP

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

London

MICHAEL Mansell (left) and Bob Weatherall of the Tasmanian Aboriginal Centre protesting on Wednesday last week outside London's Natural History Museum, which holds bones and mummified heads of some of the last members of the Tasmanian aboriginal race, which died out at the end of the last century. Echoing complaints made in the United States about the treatment of the remains of American Indians, Weatherall and his supporters object to the public display of aboriginal bones, saying that they should be returned to Australia for traditional burial.

The museum, however, is not allowed to disperse material from the collections, under the British Museums Act of 1963. Furthermore, a museum spokesman said that it "would be a tragedy" to devalue the collection by dispersal. The Tasmanian aboriginal race has died out, so these remains are the only source left for studying their origins.

Before visiting Britain, Weatherall had been to Ireland to demonstrate outside the Royal College of Surgeons in Dublin. He claims that his grandfather's mummified head was held there, and on Thursday last week the college agreed to a request from the Australian Ambassador to return the head to the Australian authorities. It has been handed over to the Australian Embassy in Ireland on the understanding that it is to be sent to the National Museum of Australia.

C.W.

Responsibilities not ducked

SIR—Your leading article about British research (*Nature* 343, 293; 1990) alleges that “departments have consistently ducked their responsibilities, notably in agriculture” for implementing the recommendation of the Rothschild report of 1971 “that government departments should stand proxy for the ultimate taxpayer beneficiaries of strategic research”.

I am afraid your leader writer has not done his homework. Rothschild said that applied research and development — R&D with the objective of practical application — should be done on a customer/contractor basis. For agriculture, the report found that a major part of the research funded by the Agricultural Research Council (ARC) was applied, that this work had no customer to commission and approve it, and that this was unsatisfactory.

It was therefore recommended that, while the Department of Education and Science should continue to fund basic and allied strategic research done by the ARC, the Ministry of Agriculture, Fisheries and Food (MAFF) should take over the role of customer for applied research.

The ministry, which already had considerable scientific expertise, therefore set up a Chief Scientists Group and took on the customer role for R&D, commissioning research specifically to meet particular needs and objectives.

Subsequent events have modified the

detail of the arrangements. For example, MAFF's focus is now very much on work that is primarily of benefit to the community at large — food safety, protection of the environment, animal welfare — as well as the strategic underpinning industry needs.

But we remain firmly committed to the customer/contractor principle and fund a substantial and varied programme of R&D. For MAFF alone this amounts to about £120 million a year. With the other agriculture departments and the Department of Education and Science the sum totals well over £200 million.

It is in the nature of our work that science and policy are closely linked. We are now working on ways of strengthening further our ‘intelligent customer’ capacity, sharpening the distinctive roles of customer and contractor in line with moves across government generally and bringing commercial views to bear more closely on the strategic research we commission.

You and your readers might like to watch out for announcements from us on these issues. I can assure you that ducking responsibilities is the last thing we have in mind.

THE BARONESS TRUMPINGTON
(Minister of State)

Ministry of Agriculture,
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Influenza causing sunspots?

SIR—Hoyle and Wickramasinghe¹ describe an association between sunspot maxima and pandemics of influenza, claiming that the relationship may be causal and that it holds over 17 cycles. In a causal relationship, it is conventional that the cause precedes the effect it produces, but in four of Hoyle and Wickramasinghe's cycles, this convention is flouted; the maxima of 1778, 1804, 1860 and 1893 follow the pandemics they are supposed to have caused.

We are not in a position to comment on the quality or accuracy of sunspot data collected in the eighteenth and nineteenth centuries, but doubt must arise about the data concerning the epidemiology of influenza. The difference between an epidemic and a pandemic is a matter of degree, and not all the dates listed by Hoyle and Wickramasinghe can be said to be those of pandemics.

Vaughan² has analysed influenza pandemics and epidemics between 1173 and 1921. Hoyle and Wickramasinghe show a pandemic in 1761–62, but Vaughan shows that this was a period of complica-

ted epidemics with poorly defined geographical distribution extending from 1757 to 1767. If Vaughan is accepted, then it is difficult to ascribe this series of epidemics to a solar event four years after its beginning.

By contrast, in 1815 influenza spread from New York to Brazil in five months, but this is not cited as a pandemic and, as in the four instances listed above, is followed by the sunspot maximum of 1816 (which Hoyle and Wickramasinghe consider to be too low to be of significance).

The name influenza probably derives from the Italian and carries telluric associations with regard to aetiology and spread. The imprecision of the epidemiological data on influenza make it difficult, at present, to establish a temporal association with solar activity and impossible to establish a causal association.

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1. Hoyle, F. & Wickramasinghe, N. *Nature* 343, 304 (1990).

2. Vaughan, W.T. *Am. J. Hyg. Monograph Ser.*, No. 1, (Baltimore, 1921).

PhD students

SIR—As a PhD student, it was with grateful relief in September last year that I found that the value of SERC (Science and Engineering Research Council) studentships had been raised by £600 (outside London) to £3,725. For me, this meant that I might be able to end my studentship next October without the incumbrance of a weighty overdraft. My bank manager says that this achievement, after six years as a full-time student through both BSc and PhD degrees, is the exception and not the rule.

There are at present several different types of studentships available, with MRC, SERC and NERC studentships at the lower end, through CASE awards to Wellcome Trust studentships at the top end. Until recently, the difference between them all has been at best £1,000 a year. I have now learned, however, that Wellcome has increased the level of studentships to the amount received by a research assistant after tax, some £7,300. Although I feel happy for those lucky few (I share a flat with one), I was left feeling disturbed by the whole affair. The Wellcome Trust makes the point that it hopes this action will prompt the government to raise publicly funded PhD studentships in the same way.

I doubt, however, that in the current political climate any such move will be made, and Wellcome's action in increasing the value of studentships is likely only further to exacerbate the tension among students on different pay scales who essentially have the same kind of motivation, responsibilities and expectations. The rise for studentships paid last year represents not a real rise in income but a readjustment of the level of the grant which has fallen by some 20 per cent since 1979.

What is perhaps most disturbing is the way in which the student loans system will affect the number of people contemplating starting a PhD in years to come. Until now, almost all studentships have been filled, indicating to the government that there is still a market demand. Ask any group of final-year biological science students whether they would like to attempt a higher degree and a large proportion will say yes. Ask them if they can afford to take such a course for a further three years, and the answer will be very different. How many will say yes to both questions in a few years' time when the financial effect of the loans scheme has bitten even more deeply? The whole system of PhD grants requires radical change before it is too late.

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The physicist and the Soviet citizen

E. L. Feinberg

Few people have had such wide and deep influence as Andrei Dmitrievich Sakharov. Here E. L. Feinberg reflects on Sakharov's life and work. On page 13 Maxim Frank-Kamenetskii recounts the circumstances of a first encounter with 'a man to remember', and on page 14 Valery Soyfer tells of Sakharov's battle against lisenkoism.

SAKHAROV — an outstanding scientist, a man of the highest moral standards, and a courageous fighter for human rights, and for a better, peaceful world. One cannot say which of these sides was predominant.

Biography

Andrei Dmitrievich Sakharov was born in Moscow on 21 May 1921, a hungry year. His father taught physics at high school and then university, and published several text books. Father, mother and no less grandmother greatly influenced his spiritual development; they belonged to the finest layer of the Russian intelligentsia and imparted its traditions to him.

Sakharov started formal education only at the age of 12, before that being taught at home with an annual examination at school. In 1938 he entered the physics faculty of Moscow University, graduating in 1942 when the university was evacuated to Ashkhabad in Middle Asia. Like all the Soviet people, he suffered from hunger during the war, and moreover went through a dangerous bout of dysentery. In 1942–44 he worked in a laboratory of a military plant on the Volga. Here he came up with four inventions for production control (one of which was patented) and, even though he had no contact with other physicists, began his research in theoretical physics. At that time he married Klavdiya Alexeevna Vikhereva, a chemist whose education in Leningrad had been interrupted by the war after four years of study. They had two daughters and a son.

Sakharov sent the results of his early researches to the well-known physicist I. E. Tamm, head of the theory department of the P. N. Lebedev Physical Institute of the USSR Academy of Sciences in Moscow. In January 1945 he became a post-graduate fellow at the institute. It was then that I first met him. Living conditions were hard; with a newly born child and a tiny grant, and without a permanent place to live (his parents' apartment had been bombed and destroyed), he helped to support his family by lecturing at the Moscow Power Institute. He defended his thesis for the degree of candidate (the equivalent of the Western doctorate) in

November 1947 and joined the department as a permanent member. After that his living conditions improved considerably.

At that time Tamm formed a small group of talented members of the staff, including Sakharov, to investigate the possibility of building a weapon based on the fusion of light nuclei. In 1948 Sakharov proposed a most important idea



which, together with another essential concept generated in the group, made a solution to the problem seem practical. Sakharov and Tamm were transferred to a special institute where Sakharov worked until 1968 (Tamm returned in 1953). Later he himself wrote that he was "the author or coauthor of several key ideas". Almost 20 years — of a period of life most valuable for any theorist — were consciously sacrificed mainly to develop and improve the weapon, although at the same time Sakharov did a great deal towards the peaceful use of nuclear fusion. He was generously rewarded by the government — with three Golden Stars of the Hero of Socialist Labour, among other orders and prizes — and in 1953 was elected a full member of the USSR Academy of Sciences. He earned the admiration and

affection of all his colleagues and of everyone else who knew about his work.

In 1961 Sakharov started to come into conflict with the government, in particular with N. S. Khrushchev, over further bomb testing. By and by, his political views were changing. In 1968 he published abroad his first famous 'manifesto' ("Meditations on Progress, Peaceful Coexistence and Intellectual Freedom") which brought him the enmity of the nation's rulers. He was dismissed from his position. Shortly afterwards, in March 1969, his wife died unexpectedly from cancer at the age of 49. This was a hard blow for him. Sakharov donated almost all of his money to an oncological hospital and to the Red Cross; he was frugal in his ways, and thus accumulated large savings. The same year he returned to Tamm's department at the Lebedev Institute with which he had renewed scientific contacts in the early 1960s, a time when he had begun to work on pure physics.

In 1972 he married Elena Georgievna Bonner, a physician, who also had years of dissident activities behind her. Persecution by the security service, and by the press and other agencies of official propaganda, was on the increase. Nevertheless Sakharov continued to have a leading part in the struggle for human rights, and in 1975 was awarded the Nobel peace prize. After his open protest against the Soviet invasion of Afghanistan he was exiled to Gor'kii and put under 24-hour surveillance by the security service. Any contact with him was forbidden (he even had no telephone), save for his wife, children and colleagues from the Lebedev Institute, of which he formally remained a member of staff. We were permitted to visit him from time to time for scientific discussions. Later his wife was sentenced to exile with him.

In December 1986 M. S. Gorbachev called from Moscow (the telephone was specially installed) and invited Sakharov to return, together with his wife, "to renew his patriotic activity". He was then free (except to travel abroad) and became a leading political figure, still trying to continue his scientific work. But there was less and less time for it, especially when in

1989 he was elected a people's deputy. His heart, which had been in bad shape even before his exile and his hunger strikes, could not withstand the strain, and on 14 December he died suddenly from a heart attack.

As was his will, Sakharov was buried in an 'ordinary' cemetery, Vostryakovo, where his first wife and the mother of his second wife had been buried. His death spiritually uplifted people throughout the country, uniting them in mourning and re-awakening the sleeping consciences of many.

Science

Throughout his life, science was an abiding passion for Sakharov. His changes of direction — from research in pure physics to technological problems, as in the period of work on nuclear fusion in 1948–68, or to humanitarian and political activity from 1968 onwards — were each time a necessary sacrifice to the urgent needs of humanity as he understood them. But at all times he tried to carry on with fundamental physics. In these conditions it is remarkable how many important ideas he put forward, although he undoubtedly did not fully realize his extraordinary talent — a talent, I should add, that was manifest equally in his work on pure physics and on its technological applications.

'Preliminary' period. Among the four papers he wrote during his time at the Volga plant, one deserves especial notice. Perhaps after reading a report on chain reactions in a uranium-moderator mixture, he realized that uranium must be prepared in blocks instead of being homogeneously mixed. This principle, which makes the chain reaction with natural uranium possible, had already been discovered but was classified.

This episode was typical of Sakharov — throughout his life he used to formulate a problem and solve it without publishing the result (maybe because he was not aware of its novelty). He called them 'amateur problems'. The subjects varied from number theory to one that attracted his interest when he helped his wife in the kitchen: in chopping cabbage there arise polygons of different sizes and with different numbers of vertices. He found, first, that the average number of vertices is four; and, second, that the ratio of the square of the average perimeter to the average area is 4π , exactly as for a circle. Solution of some of these problems is far from simple. For him they constituted a special kind of relaxation, the equivalent of playing chess.

During his postgraduate fellowship, Sakharov published three papers: on high-energy pion production, on optical determination of the temperature of hot plasmas, and on the transition between states of zero angular momentum in the nucleus. They show a sound knowledge of

the theoretical techniques of the time, and the last one — a summary of his thesis — deserves special attention because it is a fundamental contribution and bears the glittering marks of his intellect.

The fruits of this early period may seem rather modest, but one should mention that the papers were produced during only two years of work. I can testify that his extraordinary abilities emerged during numerous discussions with colleagues. The very trend of his thought was unusual. Moreover, when expressing his ideas he often used to omit some intermediate elements which seemed obvious to himself. Accordingly, at times his arguments at first seemed inconceivable and even plain wrong. Only after further, sometimes rather lengthy reasoning would it become clear that he was right.

Nuclear fusion. I have already described how Sakharov came to work on the hydrogen bomb. He himself greatly disliked being called 'the father of the Soviet thermonuclear weapon', saying that many outstanding scientists took part in the enterprise, that they put forward many of the most important ideas and solved innumerable complicated physical and technological problems. But at the same time everyone who worked on the project acknowledged his outstanding, if not leading, role.

Sakharov's work at that time was far from being limited to the weapon. His pioneering ideas laid the foundations for all further research into the peaceful use of fusion. Among them is the concept (and detailed theoretical elaboration) of the magnetic thermonuclear reactor (MTR), proposed by Sakharov and Tamm in 1950. In the MTR, both heating of an ionized gas of light atoms (deuterium and tritium) necessary to overcome mutual repulsion of nuclei, and its confinement (preventing collisions of ions with the walls of the huge toroidal 'vessel'), are effected by a system of magnetic fields. This is the 'tokamak', upon which the subsequent attempts to obtain controlled energy-producing fusion have largely centred. As proposed by Sakharov in 1951, the toroid can be covered by uranium in which neutrons liberated in the process of fusion can produce plutonium and fissionable uranium. Another way to the same end is Sakharov's idea of 'mu-catalysis'. It is possible to overcome the electrostatic repulsion of, say, deuterium and tritium without heating by using a peculiar phenomenon, first discussed in quite a different context by F. C. Frank: a negative muon in combination with deuterium or tritium forms a system akin to a hydrogen atom, but some 200 times smaller. Being neutral it is not repulsed by another nucleus and can approach it so closely that fusion becomes possible. Again, the uranium blanket can be applied, as for the tokamak. Recently a committee of

experts in the United States concluded that successes obtained with this scheme make it as promising as the traditional approaches to fusion.

Finally, the third method which is now developing — laser-induced fusion — was also first proposed by Sakharov, in a classified talk.

In 1951–52 Sakharov came up with 'magnetic cumulation', an ingenious method of obtaining extremely strong magnetic fields. The technique involves compressing a moderate magnetic field (say, within a cylinder) by 'imploding' the enveloping substance (chemical or atomic). In this way a group of his colleagues obtained record magnetic fields of 16 million gauss (in some experiments, 25 million gauss).

Return to pure physics. In the early 1960s, when his involvement in fusion was becoming less necessary, and his controversy with Khrushchev accelerated the change in his political views, Sakharov turned to pure physics. Elementary particles, field theory and cosmology were topics that had always interested him above all. The years spent on other problems had left gaps in his knowledge, however. He began to come to Moscow, to participate in Tamm's seminars more and more often, and overcame his drawbacks surprisingly quickly. His most important papers appeared after 1965.

As an example, I will describe the striking idea of the origin of so-called baryon asymmetry in the Universe; that is, why the Universe contains only matter — both baryons (protons and neutrons), and leptons (electrons and photons) — and practically no antimatter. This is surprising because in the beginning of its expansion the Universe was so hot that particles and antiparticles had to be produced in equal quantities. The answer, proposed by Sakharov in 1966, is that because of violation of CP symmetry (symmetry in respect to simultaneous change of particle charge and parity), discovered two years before, decay of a baryon is not a mirror reflection of decay of an antibaryon, and if we suppose a certain mechanism of baryon transformation the antibaryons decay faster. In a universe that is expanding fast enough, antibaryons could not survive until the present.

With extreme boldness, Sakharov proposed a hypothetical example of such a mechanism leading to instability of the proton (although with a very long life time). Such a construction seemed sheer fantasy then. But a decade later the development of the unified field theory led to the same conclusion, and the search for proton decay was proclaimed the 'experiment of the century'. Several groups have tried to find it but failed. This failure may mean that the accepted version of the unified theory must be modified. Nevertheless the entire scheme proposed by

A glimpse of Andrei Dmitrievich

I FIRST met — or, more precisely, saw — Andrei Dmitrievich in 1951 or 1952. Although I was still a child of 10 or 11, I have never forgotten that meeting.

At the time we were living far from Moscow, in an old Russian town that had been stripped of its name and was impersonally referred to (as it still is) as the 'facility', or the 'mail box'. The town itself wasn't large, and its surrounding fields and forests, rivers and villages were encircled by a ring of several rows of barbed wire and ploughed-up strips of ground. Specially trained, fierce Alsations roamed between those rows of barbed wire, and watchmen stood guard on towers.

This was the 'zone', yet another name, in this case a local one, by which it was known. People would say, 'beyond the zone', and 'inside the zone'.

But it was not a camp, or even a special research-institute-type prison. Inside that large zone there were numerous small zones where prisoners lived and worked, since everything that was built at this facility was, naturally, built by their hands. But the people who were working on the atomic project were free people — to the extent that people could be free under Stalin — who were merely involved in strictly secret work. Among those who worked on the atomic project under the direction of Khariton and Kurchatov, and under the vigilant supervision of L. P. Beria and the overall guidance of J. V. Stalin, was my father, David Albertovich Frank-Kamenetskii, a physicist.

Our family had come there almost at the very beginning, when the facility had just sprung up, in 1947 or 1948, so at the time of the events I am describing I was already a long-term resident. In

terms of living conditions, we were pretty well off. Our family had been given half of a special, two-storey, comfortable cottage. Although it was a wooden building and not very large, at the time it seemed big to me, as it did, I think, to many others. It was a luxury for those times: five rooms, a large yard and a garden where Papa raised flowers and Mama raised vegetables.

Father liked to have conversations with his fellow workers and colleagues outdoors when the weather permitted. He would lie in the hammock, his visitors would sit nearby, and they would all talk about something or other. Don't get the idea that these were seditious conversations. I think people at the time were afraid of carrying on seditious conversations even with themselves, much less with each other. (But there were exceptions. My father told me that when the 'doctor's affair' got started in 1953 he was on a business trip somewhere having something to do with the atomic project. He was lying in his hotel bed at night, and a full-length portrait of Stalin in his Generalissimo's uniform was hanging before his eyes on the wall at his feet. Father couldn't get to sleep the whole night and, gazing with hatred at the portrait, kept repeating to himself: "You ought to drop dead!". He would smile when he told about this later: "And he listened to me!".)

So, he would talk out in the yard, as I understand it, because the idiotic system of secrecy did not allow people to discuss their work anywhere away from their immediate work place. But scientists cannot help talking about their work, discussing it, thinking about it. They would call 'uranium' 'selenium' and use various other conspiratorial



tricks in order not to attract the attention of the state security officers, who were ever vigilant and ready to initiate a 'case' at any moment. In that way they would deceive them and do their work — even better if they did it in spite of Beria and his hangmen. They were convinced that they were engaged in a very important undertaking; besides, these were people who simply did not know how to work poorly.

And so once a visitor came to see my father. He was tall, very thin and ungainly, dark-haired and much younger than my father. They talked for a long time; meanwhile I was hanging around in the yard. When he left, my father, who had grown intensely pensive, called me over and said: "Remember that man. He's a genius". "Who is it?" I asked. "That's Andrei Dmitrievich Sakharov".

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Sakharov still dominates consciousness of particle physicists.

The scale of Sakharov's thinking on cosmology can be illustrated by his idea of the 'reversal of time's arrow' and of the 'multisheet Universe'. Sakharov accepted the point of view according to which expansion of the Universe is only a stage in an infinite pulsation, with many turning points (on the time axis) of maximal density of matter. For one such point he assumed that time starts to increase not only in the adjoining stage of expansion but also in the adjoining stage of 'contraction' (and therefore this is actually not a contraction but also an expansion). Here, therefore, the arrow of time is reversed. Interchanging stages of contraction and expansion are connected with each other, thus forming a 'multisheet Universe'. This hypothesis, of course, makes one giddy.

Also, there is the important work of

1967 (further developed in 1975) in which gravitation is explained as a result of quantum fluctuations of the vacuum ('induced gravity' as it is called by many authors of subsequent papers). So altogether we have three cosmological papers, based on extremely bold hypotheses, which Sakharov himself included (in 1980) in the list of his six works which he considered most important.

Still another striking hypothesis, put forward in 1984 when he was in exile, assumes that the so-called signature of metric may arbitrarily differ from the one known to us (one dimensional time plus three dimensional space); that is, signatures may differ in different parts of the Universe because of 'phase transitions of metric'. This idea has elements in common with those to be found in papers by A. Vilenkin, and by J. Hartley and S. Hawking.

Sakharov did not include in his list his 1965 paper in which formation of inhomogeneities (stars, galaxies) is explained by quantum fluctuations of metric. The idea was not upheld at that time but a decade later it found supporters. But he did include in it a series of four papers on semi-empirical formulae for baryon and meson masses based on analysis of their quark structure. This is quite a different sphere — elementary particle physics.

Political evolution

Although Sakharov was never a member either of the communist youth organization or of the party, his political views were in accordance with official ideology almost until 1956, the year Khrushchev revealed Stalin's ferocious crimes. This may seem inconceivable to those in the West but such was the case. One should take into account, first, that the 'scientific'

Against Lysenko

SAKHAROV'S name first achieved wide currency among biologists rather than physicists. These were the years of the bitter struggle against Lysenko who had set Soviet science back decades. Paradoxical as it may seem, the 'healing' of Soviet biology began from the outside, from a direction not under the control of Lysenko. The rebirth of genetics, and later of many other biological disciplines, was assisted by physicists. At this stage, Sakharov — with conviction, and with specifically sakharovian thoroughness — began his struggle against Lysenko.

In 1959 he published a large article entitled "The Radioactive Carbon of Nuclear Explosions and Nonthreshold Biological Effects" in which he criticized the view of Edward Teller, who had declared that the adverse effects of hydrogen-bomb testing were "the equivalent of smoking one cigarette twice a month". Sakharov made a precise mathematical calculation of the disturbance of the hereditary molecules from neutron action and estimated the various side effects of irradiation. In a very concise way he showed the role of mutations in the appearance of hereditary diseases, and the possibility of increase in cancer and leukaemia, of decrease in the immunological response of organisms, and of damage done to mankind because of an increase in the mutability of bacteria and viruses.

Having investigated the effect of radiation on heredity, Sakharov was able, in addition, to clarify for himself the damage being done by lysenkoism. That done, he boldly joined in the battle against Lysenko and the lysenkoites, especially against Lysenko's protégé, N. Nuzhdin. By this time Tamm and Sakharov had become legendary figures in biological circles.

It seems to me that the struggle by Andrei Dmitrievich for the interests of science was a turning point for him. It brought to light something in him that singled him out from among many colleagues: a capacity for public activity, fearlessness and adherence to the highest moral principles. In the years when he was speaking out against lysenkoism, he had not yet proved to be a fighter for the ideals of humanism — something that was to bring him world recognition. This was perhaps his first test of strength. But it was a test that clearly showed the character of this amazing man.

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structure of marxist ideology had a great appeal for Russian intellectuals. Second, experience shows that families within the Soviet Union who themselves managed to avoid becoming Stalin's victims, would remain loyal to this ideology — the twentieth century has been not so much the century of the atom or the electronics industry, as that of the realization of the immense possibilities of mass propaganda. As Machiavelli knew, a ruler must "inspire terror in such a way that if he does not deserve affection, he does avoid hate". Third, since 1948 Sakharov had been working with enthusiasm behind the curtain of secrecy. He was involved in work of the utmost importance (according to his own views) for the preservation of world peace. It was only later, when he came to realize that the nation's cynical and politically unacceptable leaders had exploited him for their own ends, and when he learned more about the suffering of ordinary people, that his views began to change. It took a rather long time. But when his mind cleared he came to a new and deep understanding, and from then nothing could stop him immersing himself in political activity.

Disarmament

It is absolutely wrong to think (as many people do) that Sakharov's political activity was a kind of repentance for the 'sin' of participation in the bomb making. At that time, as well as later, most physicists believed that the world would not be safe if one power had a monopoly on nuclear weapons. Even in 1944, Niels Bohr was greatly troubled by this, as later were Albert Einstein and Bertrand Russell. I remember Landau (who himself had been jailed under Stalin, but who nevertheless took some part in the thermonuclear project) saying to me many times in the 1960s: "Good for physicists — they saved the world from war".

Sakharov was of the same opinion. There was perhaps a moment of doubt in the period when his political views were changing. I remember a conversation with him, some time in the early 1970s. He made a remark that caused me to exclaim: "What? You regret that you took part in making the bomb?". He answered: "You know, there are certain questions about which it is better not to think too much". But this doubt was temporary. Later his struggle for disarmament was intensified because of the danger of the accumulation of stocks of nuclear weapons and of their proliferation. I heard at second hand that during his meeting with Edward Teller about one year ago he said to him something of the kind: "Essentially, you and I were solving a common problem". On another occasion I myself heard him say, "Teller is a tragic figure".

Sakharov's belief was that one should struggle for non-proliferation, and for

reduction of nuclear arsenals as well as of conventional armaments. But he saw this as being possible only in the open world with human rights truly secured. In sum, I am sure that his political and social activities would have followed the same course even if he had not been involved in the making of the bomb.

Human rights

The great significance of Sakharov's struggle for human rights lies not so much in the practical results of dissidence as in its tremendous moral and spiritual effect. For the first time in many decades people of my country saw a man of extreme honesty, sincerity and nobility who fearlessly faced the state machine — both in proclaiming large-scale political ideas and in defending individuals. He was devoted to his fellow fighters for human rights. He was ready to struggle for every one of them, and never missed a chance to call attention to those who were in jail or exile. The names of such people were included in his Nobel lecture and in his letters to the government. When Gorbachev told him that he was free he immediately responded that all of them also should be liberated. When later he was told that his honours (of which he had been deprived when sent to exile) were to be returned to him, he refused to take them back until all dissidents were rehabilitated.

This indivisible combination of deep feelings for humanity as a whole and for single individuals was the most remarkable feature of Sakharov's personality. Together with his absolute inability to say anything that did not correspond with his convictions, with what he actually thought and felt, it explains why both ordinary people and the political leaders of the world listened to and trusted him. At the same time he did not claim to be a deity. Once, in the middle of the 1970s, I said to him: "You know, Andrei Dmitrievich, some of your admirers nevertheless believe that neither yourself or Solzhenitzyn should give recommendations concerning concrete political and economic problems since you are not professionals". He exclaimed: "Of course, I am not a professional, of course, I make errors. But what am I to do if nobody else can or dares to say a word?"

A year or so ago, I asked him whether certain recollections about his younger years were true. He became indignant: "All this is a myth to make a superman of me. I am a man like others". It would be unjust to suspect that here he was posturing; he simply meant that although he knew his worth, he did not want to feel himself to be apart from other people. And he was not. We have lost one of the finest examples of humanity. □

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Cosmic rays lose dramatic quality

The discovery of cosmic rays was a dramatic event early in this century, but the resolution of the problems then posed is turning out to be a quieter (but nonetheless interesting) affair.

THE question of where cosmic rays come from has been with us for most of this century, ever since C. T. R. Wilson's device for simulating the formation of clouds in the laboratory happened also to reveal a previously unsuspected flux of charged particles of whose origin the only certainty was that it could not lie in the Solar System. For much of the time since then, the origin of cosmic rays has been as much in doubt as at the beginning. But now there seems to be a chance — within the foreseeable future, perhaps by the end of the century — that the issue will be so clarified that many will regard it as settled.

The reasons why the cosmic-ray problem is so difficult are so plain that they are most often overlooked. The most obvious, and thus the most distracting, is the sequence of processes by which whatever particles constitute the primary flux of cosmic rays are successively converted into others. There is, for example, the atmosphere of the Earth, which ensures that all particles but a sizeable proportion of neutrons and all neutrinos, will be converted into other mixtures of the constituents of the primary flux. But the stream of particles reaching the top of the Earth's atmosphere will have reached there only by travelling at relativistic speed through interstellar matter, interacting (among other things) with nuclei to produce the nuclear fragments recognized over the past thirty years as significant components of the cosmic-ray flux collectable by balloons.

The Earth's atmosphere and interstellar matter are but two of the screens standing between observers and the true primary flux.

By any reckoning, the primary particles — whatever they are and wherever they come from — travel the Galaxy for intervals of time comparable with its age, so that there is plenty of opportunity for even the least likely interactions between cosmic-ray particles and others to set their stamp on the composition of the cosmic rays eventually observed. If, as now seems likely, at least some primary-cosmic ray particles come from other galaxies than our own, their interaction with the ubiquitous microwave radiation background must also be taken into account.

That interaction is, for example, one of the distinctive features of an article by J. Wdowczyk, from the Institute of Nuclear Studies at Lodz in Poland, and A.W. Wolfendale, from the University of Durham, in the United Kingdom (*Astro-*

phys J. **349**, 35-40; 1990), which is chiefly concerned with spelling out tests of the possibility that a fraction of primary cosmic-ray particles may be extragalactic, but which is in passing a neat summary of how the conundrum of the origin of cosmic rays has been particularized into a manageable string of apparently answerable questions.

The issue of whether cosmic rays are confined to the Galaxy (and, presumably, to other galaxies as well) or are a supragalactic phenomenon has not been much debated since the 1950s. Plainly, the flux of energy in the cosmic rays reaching the surface of the Earth cannot be representative of the Universe as a whole, for the amounts of energy involved are comparable with those locked up in the cosmic microwave radiation background — itself a substantial fraction of the energy of the Universe in any view of the mechanism of its formation. If cosmic rays were truly ubiquitous, it would be necessary on those grounds alone to rewrite the "Big Bang" script — but the cosmic rays would also interact with the background radiation so effectively that the properties of both would be transformed.

But if the distribution of cosmic rays cannot be uniform throughout the Universe (so that their origin cannot lie in some event characteristic of the whole Universe rather than of its parts), it is unthinkable that all galaxies would be impervious to the escape of a fraction of the cosmic-ray fluxes which they contain. So there will be some intergalactic flux of cosmic-ray particles, where that term must be taken to include γ -rays as well as protons, neutrons and electrons. As things stand, nobody can be in a position to tell how the present leakage of cosmic rays from galaxies compares with the creation of new volume in the Universe on account of its expansion.

The other long-standing conundrum is the mechanism by which particles are accelerated to high energy. Since the 1920s, the goalposts have been moved repeatedly, in such a way that the gap between them has been steadily enlarged, chiefly by the development of techniques for the measurement of the energy of energetic particles.

In retrospect, this has been a curiously empirical process. From the outset, it must have been plain that measures of the energy of a particle provided by the thickness of lead or other metal plate it would traverse would be at once rough and ready

and incapable of providing information about the most energetic particles of all. Plainly, there are also (and will remain) limitations on what can be done by estimates of the energy of charged particles among the cosmic rays by their deflection by powerful magnetic fields; of necessity, instruments to accomplish that would have to be comparable in size with the scale on which particle accelerators are constructed, but the interest in cosmic-ray physics and because substantial part of the primary flux consists of particles which are several orders of magnitude more energetic than can be produced by accelerators.

Wdowczyk and Wolfendale take the view that there is nothing in the measurements to suggest a fixed upper limit on the energy of cosmic rays. Extensive showers of secondary particles in the Earth's atmosphere have been able to verify that there are primary particles with energies as great as 10^{20} electron-volts (eV), but that is largely a function of the size of shower detectors so far constructed. Whether larger and more sensitive detectors will uncover the footprints of still more energetic particles will depend on whether the underlying processes that generate the energetic cosmic rays are themselves inherently limited in the energy they can impart to particles.

So where do these energetic particles come from? The passage of time has made this seemingly crucial question seem less and less important. But just as it may be unreasonable to expect a black-and-white answer to the question as to whether cosmic rays are a galactic or extragalactic phenomenon, so it is likely that there is no unique source of cosmic rays. In some respect, that must be so. Some of the particles flung off from the Sun in the course of solar flares, for example, must eventually contribute to the general flux of cosmic rays. Other main-sequence stars will presumably behave in the same way, but active neutron stars and supernovae (past as well as present) will be more prolific sources of more energetic particles. Yet objects such as those do not account for the most energetic particles of all — whence interest in what are called γ -ray bursts, whatever they may be. The conclusion that there is no unique source may of course be a disappointment. How can such a dramatic discovery as that of cosmic rays have such an anticlimactic denouement? That, sadly, is often how it is.

John Maddox

Nosing ahead in the cold war

Charles R.M. Bangham and Andrew J. McMichael

As viruses are intracellular parasites, the ideal anti-viral agent would prevent entry of the virus into a potential host cell, so minimizing the tissue damage caused by the virus and by the immune response to it. Most existing anti-viral agents, such as α -interferon, acyclovir, AZT and ribavirin, act only after penetration of the cell by the virus. But on page 70 of this issue, Marlin *et al.*¹ describe a novel method of preventing infection of cells by the common cold virus (rhinovirus) using an agent that targets the virus 'receptor' — the cell-surface molecule that binds the virus and allows its entry into the cell.

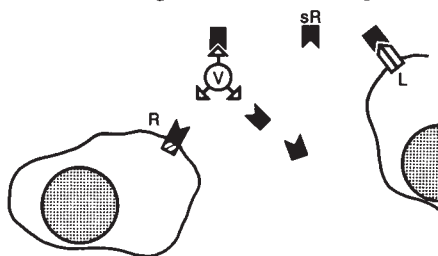
The recent identification of the cell-surface receptors for Epstein-Barr virus², HIV (refs. 3, 4), poliovirus⁵, rhinovirus^{6,7} and the murine ecotropic leukaemia virus⁸ has focused research on receptor targeting as a strategy for new anti-viral drugs. In particular, the discovery that the CD4 molecule is the main cellular receptor for HIV (refs 3, 4) has raised hopes of using either soluble CD4 (ref. 9) or a CD4-Fc chimaeric molecule¹⁰ to prevent infection by the AIDS virus. Marlin *et al.*, one of the groups that identified⁷ ICAM-1 — intercellular adhesion molecule-1 — as the main receptor for human rhinoviruses, have now used this principle to block rhinovirus infection of cells *in vitro*.

They have produced a soluble form of ICAM-1 (sICAM-1) by introducing a stop codon just outside its predicted membrane-spanning region. They then transfected this mutated gene into Chinese hamster ovary cells, amplified it by dihydrofolate reductase/methotrexate selection, and selected those clones that produced large amounts of sICAM-1. The immunoaffinity-purified molecule retained the ability to bind three anti-ICAM-1 monoclonal antibodies, indicating that the structure of the soluble molecule was at least broadly similar to the membrane-bound (native) form. sICAM-1 specifically inhibited the cytopathic effect induced by a member of the major rhinovirus subgroup (HRV54), but did not moderate the effect induced by non-ICAM-1-binding rhinoviruses (of the minor subgroup), other picornaviruses or the unrelated herpes simplex virus (HSV-1). sICAM-1 also inhibited binding to cells of radiolabelled rhinovirus particles in a dose-dependent manner.

The structure of picornaviruses (which include rhinoviruses) is known to a high level of resolution¹¹. There is some evidence¹², as yet unproved, that deep pits or 'canyons' in the surface of the virion contain the receptor-binding domains of the virus. If such receptor-binding canyons

are inaccessible to antibodies, as their dimensions indicate, this could explain the failure of antibodies against one rhinovirus strain to neutralize another strain¹¹. The protein sequence in the canyon wall is highly conserved, presumably because there is strong selective pressure to maintain the receptor-binding capacity. Soluble receptor molecules (or their analogues) should have the virtue that they attack this highly-conserved Achilles' heel of the virus, which other anti-viral agents cannot reach.

Marlin *et al.* mention the possibility of using sICAM-1 or an analogue as an anti-viral drug. But several important



The virus particle (V) adsorbs to a cell by binding to the cell-surface receptor (R). The soluble receptor analogue (sR), made by deleting the transmembrane and cytoplasmic domains of the receptor molecule, binds to the virion and so prevents adsorption of the virus to the cell. If the receptor is an adhesion molecule, the soluble receptor analogue will also bind to its ligand (L), and so may interfere with normal cell-cell interactions.

hurdles will have to be surmounted before such a drug could be used in humans. First, the efficacy of the soluble receptor analogue must be demonstrated *in vivo* in an animal model; it must be efficient in concentrations that can be achieved and maintained on the nasal mucosa. Second, frequent doses of any substance, especially a protein, may cause sensitization in the nasal mucosa, inducing an immediate (anaphylactic) immune response, or an immune-complex-mediated hypersensitivity response such as pituitary snuff takers' lung. If a close analogue of a native protein is used, autoreactive antibodies might also be produced.

Third, as Marlin *et al.* observe, the effects of soluble receptor analogues on normal cell-cell interactions must be studied. This is particularly important when the virus receptor is an adhesion molecule responsible for binding to a specific ligand on another cell. Intriguingly, three of the recently described cell-surface receptors (those for HIV, poliovirus and rhinovirus) are members of the immunoglobulin 'superfamily'¹³ of proteins, the largest known family of cell-adhesion molecules. We know a little

about the immunological importance of the interaction of ICAM-1 with its ligand, LFA-1, from the study of patients with congenital deficiency of LFA-1 (ref. 14). Such patients suffer from indolent bacterial and fungal infections and most die before the age of two years. But these patients usually have a defect in the β -chain of the LFA family (CD18), and so lose the function not only of LFA-1 but also of two related glycoproteins (CD11b and CD11c). As these do not bind to ICAM-1, blocking the function of ICAM-1 with sICAM-1 may have less effect. Furthermore, LFA-1 is known to interact with at least one other ligand, ICAM-2 (ref. 15).

These results will also encourage efforts to clone the cell-surface receptors of other viruses, both to develop similar anti-viral drugs and to understand better the tissue specificity and infectivity of certain viruses. A typical strategy has involved antibody blocking of virus binding (of rhinovirus and HIV for example), but this depends on large panels of monoclonal antibodies (which are incomplete), and has not been uniformly successful. New approaches are needed. These might include expression cloning systems¹⁶, whereby the putative receptor-binding protein of the virus, such as the envelope protein of retroviruses, is used as a ligand to select transfected cells expressing the receptor molecule. This could lead directly to cloning of the receptor, unless it is a complex protein encoded by two distinct messenger RNAs.

The success of receptor analogues as anti-viral drugs will of course also depend on whether the virus can enter the cell by other routes. But the identification of virus-receptor molecules has opened a new age in virology. At the least, we shall understand more of the behaviour and evolution of viruses; at best, it makes possible a new approach to the rational design of anti-viral drugs. □

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The rough with the smooth

Philip Ball

THE behaviour of solid surfaces is anything but superficial. For those interested in the states of matter, surfaces and thin layers deposited on them make available a whole new set of phases, whose behaviour, being essentially two- rather than three-dimensional, differs markedly from that of bulk states. A clutch of papers appearing consecutively in *Physical Review Letters*¹⁻⁵ share in common a concern with transitions between surface phases.

The study of melting, for example, has become focused on processes at the surface, because that is where, in many cases, the bulk transition seems to be initiated^{6,7}. One question that arises is whether surface melting is related to the phenomenon of roughening, which has been found to precede it⁷.

A flat surface becomes progressively rough with increasing temperature, as relatively mobile surface atoms migrate to form steps and other defects. Theory⁸ and experiment⁹ show that there exists a roughening transition at temperature T_R , below which small surface irregularities contribute to an interfacial region of finite thickness between bulk solid and the vapour or vacuum above the surface. At T_R , the amplitude of these surface fluctuations increases abruptly and the interfacial width becomes infinitely thick. In real systems, the transition can be identified by a change in the character of solid nucleation at the surface — this occurs isotropically above T_R , whereas below the transition one finds growth of faceted crystallites.

Cao and Conrad⁴ have investigated the onset of thermal roughening using low-energy electron diffraction to follow changes in the vibrations of atoms at a nickel surface (specifically, the surface defined crystallographically as (110)).

The electron-scattering data are consistent with the presence of a roughening transition somewhere in the region of 1,300 K, which is about three-quarters of the bulk melting temperature. But the roughening seems to be a two-step process, because a marked change in scattering is first seen at about 900 K. Cao and Conrad attribute this change to the development of a pronounced anharmonicity in the Ni-Ni vibration at the surface.

This anharmonicity probably drives the onset of roughening. The anharmonic vibrations result in an average Ni-Ni distance in the surface layer that differs from that in the bulk, and thus there is a build-up of stress in the surface layer. This layer lowers its free energy by forming vacancies, thus reducing its density. As the temperature increases, so does the

vacancy concentration; ultimately the vacancies cluster to form stepped regions, and roughening results.

Clustering of surface defects is also observed by Shen *et al.*⁵, although in this case the defects are steps that have been created artificially by miscutting a Ni(111) surface at an angle of 5°. The authors use X-ray diffraction to probe the average step height as a function of temperature, and find that it decreases suddenly at around 570 K. They attribute this to clustering of the steps below this temperature. Although the details of the surface phases are rather different from those of Cao and



Cross-sectional profile of the solid-on-solid (SOS) model for surface roughening. The surface comprises stacks of atoms; the insets show forbidden configurations (inclusions and overhangs). At the roughening transition temperature, the average width of the interface, W , diverges logarithmically.

Conrad, the results are consistent in so far as they support an attractive interaction between surface irregularities.

Roughening has been studied extensively using lattice models, which are a good approximation to a regular crystal structure. The most tractable of these are solid-on-solid (SOS) models^{8,10}, in which the surface comprises stacks of atoms of different heights — surface atoms cannot sit above vacancies and overhangs are forbidden (see figure). In the simple SOS model only nearest neighbours interact, and there is a roughening transition of a type specific to two-dimensional phases (a Kosterlitz-Thouless transition)⁸.

den Nijs¹ considers an extension of the SOS model in which next-nearest-neighbour interactions are included, and nearest-neighbour stacks are allowed to differ in height by no more than one atom. For this system, the phase diagram is more complicated. For some values of the various interaction strengths there is a simple roughening transition, but for others roughening is a two-stage process: the 'flat' and 'rough' states are separated by an intervening phase in which the interfacial width is still finite (that is, the surface is not yet rough) but the steps exhibit no long-range order. den Nijs calls the transition from the ordered flat to

disordered flat phase 'pre-roughening', and associates it with vanishing of the free energy of step formation. Pre-roughening has not yet been observed experimentally, but den Nijs identifies Ag(110) and Pd(110) surfaces as promising candidates.

Clearly, surface restructuring can be a complex process; but when an adsorbed phase is introduced, the phase diagram is enriched still further, as illustrated by Youn and Hess³ in their study of oxygen films adsorbed on graphite. Phase transitions in the film can be placed in two categories: those between successive adsorbed layers (layering transitions and wetting), which tend to be reflected in changes normal to the surface, and those within the layers (melting and commensurate-incommensurate transitions), in which the changes tend to be parallel to the surface. Youn and Hess use ellipsometry to map out the phase diagram of this system in detail.

The interesting region lies between 37 and 55 K. At about 44 K, bulk oxygen undergoes a solid-solid transition from the β to the γ form; at 54.35 K, bulk γ -solid, gas and liquid coexist at the triple point T_t . As one might expect, these bulk transitions influence the surface behaviour.

A key issue is whether the surface-adsorbed phase 'wets' the substrate. If the surface film — which is solid (rigid) well below T_t but liquid (mobile) above it — becomes infinitely (that is, macroscopically) thick as the oxygen vapour pressure approaches saturation, there is complete wetting, whereas a finite film thickness implies partial wetting. Youn and Hess find that below 43.8 K the graphite is completely wetted by a β -like solid film which grows via a sequence of transitions that increase the thickness by one molecular layer. But between 43.8 K and T_t there is partial wetting — the film growth terminates at finite thickness and three-dimensional islands of γ -solid appear. At T_t there is 'triple-point wetting' by a liquid-like film, although even above T_t the liquid film grows initially in a sequence of layering transitions.

Youn and Hess find that the melting transitions between solid and liquid films are far from simple, because melting of individual layers can occur in multilayer films. For example, the transition from a three-layer solid film to a three-layer liquid film occurs not in a single step but first by melting of the topmost layer and then by simultaneous melting of the other two. Furthermore, melting in two dimensions can be either first order (for which there is a heat of fusion) or higher order (continuous). Here the latter pertains only for the topmost layer.

Some of these features are found for other adsorbed films, for example ethylene¹¹ and methane¹² on graphite, but there seems to be no generic phase

diagram. It is clear, though, that bulk transitions act to 'pin' the asymptotic behaviour of some surface phenomena. Parry and Evans², on the other hand, have looked at the effect of a surface transition (wetting) on bulk-like behaviour (liquid-gas criticality). They have studied the phase equilibria of a fluid confined between two planar, parallel surfaces with attractive wall-fluid forces. This system has been used to model gas adsorption in porous substrates such as carbon blacks, in which the pores have this kind of slit-like geometry¹³. In general, the confined fluid has a transition between liquid-like and gas-like states, but the pressures and temperatures at which the states coexist are shifted relative to the bulk system. The shift of the pore critical point, $T_{c,L}$, at which the states become indistinguishable, relative to the bulk T_c depends only on the separation of the walls, L , and the bulk critical exponent ν (ref. 14).

Parry and Evans consider how this situation is modified when the walls are not identical. Specifically, wall 1 is strongly attractive, favouring the filling of the pore with liquid, whereas wall 2 has a net effect of favouring the gas-like phase. Thus this asymmetric arrangement can be considered to 'frustrate' the fluid. In their particular case, a wetting transition and a drying (wetting by vapour) transition would occur at walls 1 and 2 respectively, for infinite L , at the same temperature T_w . The phase diagram for these asymmetric pores still contains a two-phase region terminating in a capillary critical point $T_{c,L}$, but this is now controlled by T_w rather than T_c , in the sense that as $L \rightarrow \infty$, $T_{c,L} \rightarrow T_w$ rather than T_c , and the exponent is that of the (continuous) wetting transition. Thus the wetting behaviour here affects the critical behaviour qualitatively, causing the fluid to 'forget' about bulk criticality and become dominated by the surface equilibria. □

Philip Ball is an assistant editor of *Nature*.

Distorted views of the Universe

Daniel W. Weedman

THE ability of massive astronomical bodies to bend the paths of light is an increasing source of fascination. In part, this is because of the diverse manifestations of such gravitational lenses. An exotic example is given by Ostriker and Vietri on page 45 of this issue¹, who suggest that lenses may be responsible for the occasional illusion by which distant quasars appear to be embedded in nearby galaxies. But also, there is the prospect that large invisible masses, perhaps including new forms of matter, may be revealed by their lensing effect.

Gravitational lensing was first observed by an international group of astronomers in 1919, who found that the positions of stars on the other side of the Sun, viewed during a solar eclipse, were marginally

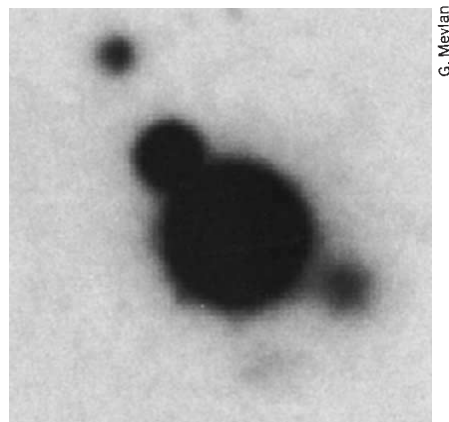
multiple images or not.

Naturally, the question arises: are they images of a single quasar or are they in fact distinct quasars? The way to tell is to compare their spectra. Reporting on their latest results at a recent meeting², Meylan, Djorgovski and Shaver of the ESO/Palomar collaboration described two null results of considerable interest. Using this method they identified a new pair of quasars, PHL1222, with very little separation (see figure). The spectroscopic differences are sufficient for the authors to conclude that the components are distinct quasars, but probably so close, perhaps as members of a galaxy cluster, that they are interacting. Astrophysicists who believe that quasars are triggered during galaxy-galaxy collisions³ are likely to be encouraged by this observation.

The collaboration also reported on an investigation of the quasar pair 2345+007, separated by several seconds of arc, for which no lensing galaxy has been found. The authors used computerized image-enhancement methods to analyse the shape of each component in an effort to find structure at scales below the image resolution. The results were inconclusive, but had the pair been lensed, a third image should have been apparent: none was. In this case at least, there is as yet no compelling requirement to invoke a lens with mysterious properties.

BL Lacertae objects are galaxies that contain bright, variable compact sources that resemble quasars in many ways. In this issue, Ostriker and Vietri¹ use recent data to strengthen their earlier suggestion that many BL Lac objects are in fact artefacts of microlensing, which arises from the gravitational lensing by individual stars within a galaxy. Microlensing works only if the lensed object is very small, as are the sources of continuum spectra in quasars. The distinguishing characteristic of BL Lac objects is the intense continuum spectrum which lacks emission lines. Thus microlensing of quasars fits the bill, as their line emission arises in larger volumes. The rapid variability of BL Lac sources can be related to the passage of the lensing stars across the line of sight to the quasar.

The authors also suggest that microlensing can be compounded by the image intensification arising from the distributed mass of the intervening galaxy to produce the tendency for quasars at high redshift (very distant) to be in close proximity to nearby (low-redshift) galaxies. This is similar to the explanation for Huchra's object, a nearby galaxy with a multi-component high-redshift quasar at its centre. Such objects have occasionally



G. Meylan

The two central quasars of PHL1222 are barely separated, but are not the lensed images of a single source. The proximity of other extended sources suggests that the quasars are in a cluster of galaxies formed less than 3×10^9 years after the Big Bang.

displaced. This constituted partial experimental proof of Einstein's theory of general relativity, which predicted that the gravitational effect of large masses could bend rays of light. The most celebrated lenses, typically massive galaxies, are responsible for multiple images — doubles, quartets and even 'Einstein' rings — of distant quasars.

An important question is whether the lensing of a quasar can always be associated with an observable galaxy, or whether the massive object in some cases is invisible, perhaps indicating the existence of some exotic matter. One group addressing this question has been using the telescopes at the European Southern Observatory (ESO) in Chile and at Mount Palomar to conduct a systematic search for lensed quasars. Candidates for examination can be found by identifying quasars that are unusually bright — lensing also intensifies images. Careful observation can reveal whether the object comprises

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been cited to support the contention that the redshifts of quasars are not cosmological in origin, which is to say that they do not indicate the great distance to the quasars. Microlensing provides a more satisfying explanation because it predicts many of the details seen in the observations.

An Einstein ring is the image predicted to be generated if a source of light is precisely behind a lensing mass with simple structure. On page 43 of this issue¹, Langston *et al.* discuss the most elegant such ring, the image of one of the radio lobes of the quasar MG1654+1346 magnified by an intervening galaxy (see cover). From detailed observations of the ring, Langston *et al.* show that the quasar's radio lobe has three components, a region of diffuse emission overlaid by two hotspots. They also obtain a measure of the lensing galaxy's mass, around 3×10^{11} solar masses, although allowance must be made for the uncertain distance to the galaxy.

Similar distortions of extended background objects produce arcs of light when a distant galaxy is distorted and magnified by an intervening galaxy cluster acting as a lens. An extraordinary demonstration of this effect is shown in a recent paper by

Tyson, Valdes and Wenk⁵. They show faint images of many background galaxies, each of which is only slightly distorted, but which together form portions of circles centred on the lens. The results are notable because of the faintness of the galaxies seen, and because of the conclusion that the distribution of dark matter in the clusters is similar to that of the visible cluster galaxies.

Rarely in astronomy can such a simple theory as gravitational lensing be applied to such a wide range of observations, many of great complexity. The bizarre manifestations of these lenses should continue to provide otherwise unobtainable information about dark matter in the Universe. □

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IMMUNOLOGY

Making a molecular match

Ronald N. Germain

MOST T lymphocytes initiate immune responses upon specific recognition of antigen-derived peptide bound to highly polymorphic cell-surface class I or class II major histocompatibility complex (MHC)-encoded molecules. The immune potential of the T cells of an individual therefore depends on (1) the range of antigen-derived peptides that can effectively be bound by the specific allelic forms of the individual's MHC molecules and (2) the capacity of the set of clonally distributed T-cell $\alpha\beta$ receptors (TCRs) to bind these peptide-MHC complexes and initiate T-cell activation. Two papers, one by Nikolić-Žugić and Bevan on the influence of class I MHC structure on thymocyte selection (see page 65 of this issue¹), the other by Garrett *et al.*² on the comparative crystallographic structure of two human class I MHC molecules, suggest a model for the molecular matching of the repertoire of peripheral receptors with the set of foreign peptide-self-MHC complexes likely to be encountered during an individual's lifetime.

T cells populating peripheral lymphoid organs have a bias towards the recognition of peptides presented by the allelic forms of class I and class II MHC molecules encountered during development in the thymus, specifically those expressed on stromal cells resistant to radiation³. Because the loci containing the rearrang-

ing elements encoding the α and β chains of the TCR are not genetically linked to the MHC, such a bias must arise by selection of individual clones of cells after the generation of an initially allele-neutral repertoire in early thymocytes. For many years it was assumed that such thymic selection involved the survival and maturation of thymocytes with receptors having a moderate but definite affinity for the unique structural features of self-MHC molecules alone. Given the current view that essentially all MHC molecules expressed on the cell membrane are occupied with either a foreign or self peptide, models for this positive selection segregate into three categories — those that admit to the presence of self peptides in stromal MHC molecules, but which argue that these peptides do not contribute significantly to the specific binding involved in selection; those suggesting that the binding of both self peptides and polymorphic MHC structures is important to recognition events in selection; and those postulating that it is only the bound peptide, and not the MHC molecule itself, that contributes to the binding events underlying selection. In this last case, the bias for allele-specific self-MHC structure results only from the influence of such MHC structural variation on the binding of peptides for presentation⁴.

The radiation chimera experiments of

Nikolić-Žugić and Bevan provide strong, if indirect, evidence that self peptides bound to MHC molecules are important in positive selection, which is consistent with the last two models. These investigators studied the influence of variation in the structure of the class I molecule K^b on selection of a repertoire of T cells able to respond to an ovalbumin (OVA) peptide plus K^b (ref. 5). They found that T cells developing in the presence of the K^{bm8} mutant class I molecule expressed on radiation-resistant thymic stroma have no OVA- K^b -specific T cells in the periphery. Because they showed that the chimaeras have active K^b -presenting cells that should allow priming of the OVA- K^b response, and because self-tolerance does not account for the failure of the mice to respond, they concluded that the effect of the K^{bm8} mutation is to prevent positive selection of the OVA- K^b repertoire. The differences between K^{bm8} and wild-type K^b proteins are found only in the floor (β strands) of the predicted class I MHC molecule peptide-binding groove^{6,7}. Serological evidence and computer modelling based on the HLA crystal structure both indicate that these changes should not affect the conformation of the rest of the class I molecule, especially not in sites accessible to direct TCR binding. An obvious implication is that a change in the set of self peptides bound to stromal cell class I molecules as a result of the $bm8$ mutation accounts for the failure to positively select the OVA- K^b repertoire. This is consistent with a role for non-MHC gene products in positive selection⁸, or for MHC molecules other than the stromal-selecting element, presumably because they provide the critical self peptide⁹.

Unexpected connection

Two other observations in the report¹ provide exciting new information about how self peptides contribute to the selection process and on the relationship between self peptide-based selection and peripheral antigen recognition. First, the authors note that, in addition to failing to select an OVA- K^b -specific repertoire *in vivo*, the K^{bm8} molecule cannot present the OVA peptide to OVA- K^b -specific T cells *in vitro*, which might reflect the absence of OVA peptide binding to the K^{bm8} molecule. This implies that there is an unexpected connection between (1) the failure to bind a specific self peptide in the thymus, (2) the inability of the same MHC molecule to bind a foreign peptide, and (3) the lack of mature cells able to recognize that foreign peptide, even when it is effectively complexed with an MHC molecule (K^b in this case). The second observation is that there is an effective repertoire for a different antigen presented by K^b (vesicular stomatitis virus (VSV) nucleocapsid protein) in mice whose T cells have been selected on K^{bm8} ,

indicating that the effect of the bm8 mutation on repertoire selection is antigen-specific.

These observations are notable in light of results from Garrett *et al.*, who have reported the high-resolution crystal structure of two closely related human class I MHC molecules, HLA-A2 and HLA-Aw68 (ref. 2; see also the News and Views article on page 617 of the same issue). It is striking that there are actually binding pockets within the putative peptide-binding groove of these molecules, whose accessibility depends on residues showing allelic polymorphism. This might explain why there is a common structural motif in many but not all peptides that bind a given MHC molecule¹⁰ — those

sharing the motif could bind to a major subsite, whereas those without it might bind at one or more distinct subsites. Furthermore, if one presumes that distinct peptides are more dependent on one subsite rather than another for their binding, then two MHC molecules that differ in some subsites, but share others, would bind some foreign peptides in common and differ in the binding of others. This would fit nicely with the differential effects of the bm8 mutation on presentation of OVA versus VSV. The experiments of Nikolić-Žugić also suggest that the K^b and K^{bm8} molecules vary in their binding of self peptides in a similar way and that there is a relatively strict relationship between the set of self peptides

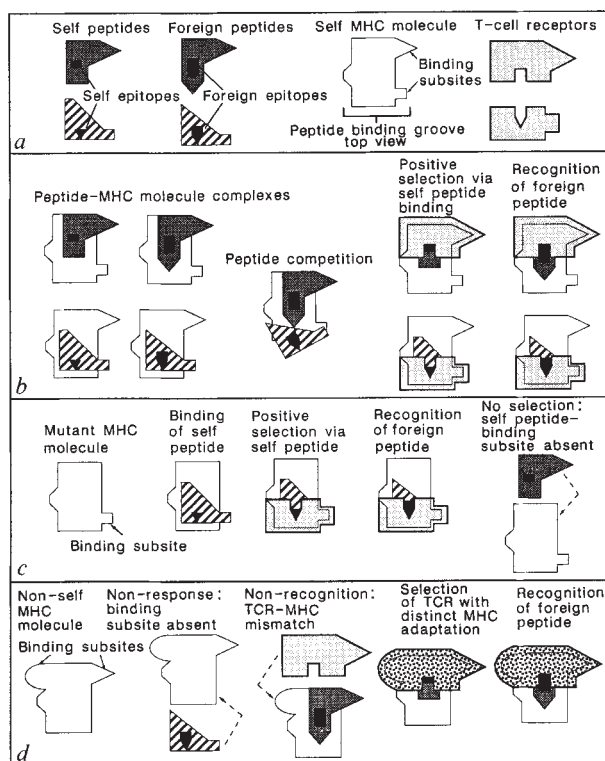
bound at a given site and the ability of mature T cells to respond to foreign peptides bound in the same subsite. The existence of a structural relationship between thymic selecting peptides and foreign-peptide recognition was in fact proposed several years ago⁹.

Evolutionary context

What could this relationship be and does it involve TCR–MHC interactions as well as peptide recognition? To answer these questions, it is important to consider the phenomenon of positive selection in the context of the evolutionary accumulation of MHC molecule polymorphism. It makes the most sense for the immune system to fill secondary lymphoid tissue with only those cells that can potentially function in the individual, to maximize the frequency of individual clonal specificities. Thus, for optimal activity, it is reasonable for peripheral T cells to be biased towards the recognition of peptides that can associate with the available (self) MHC molecules. This requirement does not necessitate direct attention by TCRs to the polymorphic structure of the MHC molecule itself. In fact, if successful TCR–peptide interaction demands any TCR–MHC contact at all, it would be least wasteful to maintain in a conserved state those regions of the MHC molecule able to contact the TCR, altering only those sites that uniquely affect peptide binding. This strategy would maximize the usable fraction of receptors among those created by gene rearrangement, as no receptor would be excluded from selection because of a failure to interact with the available MHC molecules. Consistent with this view is the finding that, using the HLA-A2 crystal structure as a model, no highly polymorphic positions are found among those residues whose side chains would be orientated away from the putative peptide-binding site and exposed for pure TCR interaction^{7,11}. This argues that MHC mutations are fixed in the population only if they contribute to diversity or strength of peptide binding, independently of any potential for altered interaction with the TCR. It also argues that direct recognition of polymorphic MHC structures is unlikely to be the primary driving force in positive selection of the T-cell repertoire. The simplest model for positive selection would therefore involve the continued maturation of thymocytes bearing TCRs that recognize only the self-peptide portion of peptide–self-MHC complexes⁴. These TCRs would presumably cross-react widely with the structurally related set of foreign peptides able to occupy the particular binding sites of that individual's MHC molecules, thus providing a suitable repertoire.

By analogy of the TCR interaction with a peptide–MHC complex to the binding of immunoglobulin with protein antigens,

Molecular relationship between positive intra-thymic selection and peripheral antigen recognition. *a*, The self and foreign peptide pairs shown each share structural similarity in the regions controlling binding to MHC molecules and the regions contacting the TCR (epitopes). The self MHC molecule has binding subsites (pockets) suitable for both sets of peptides. The TCR can accommodate the self MHC molecule and each has the ability to bind one of the related self and foreign peptide–epitope pairs when aligned with the MHC structure. The MHC molecule is drawn as the outline of the peptide-binding cleft seen from the top of the molecule, as shown in Fig. 3c of Garrett *et al.*². *b*, This shows each peptide bound to the MHC molecule, the recognition by each TCR of the self peptide–MHC molecule complex leading to positive selection, and the subsequent recognition of the related foreign peptide–MHC molecule complex. Only the TCR selected on a self peptide occupying a given binding subsite of the MHC molecule can participate in recognition of the foreign peptide occupying the same subsite, because of constraints on TCR–MHC interaction and the limitations on peptide epitope location dictated by the binding subsite being used. Competition for the peptide-binding groove¹⁸ would be observed between two peptides binding to distinct subsites. *c*, The mutant MHC molecule shown here is identical to that in *a* and *b*, except for the loss of a binding subsite. This represents the fundamental difference between the K^b and K^{bm8} molecules studied by Nikolić-Žugić and Bevan¹. This molecule still binds one of the two self peptides involved in positive selection, allowing the generation of a repertoire to the related foreign peptide (for example, VSV nucleocapsid). The absence of the second binding subsite prevents the presentation of the other self peptide, precluding selection of the second set of TCRs, including that involved in responses to the second foreign peptide (such as OVA). *d*, A non-self MHC molecule is shown, which lacks one of the binding subsites of the original molecule and also has structural variations in TCR accessible regions (for example, K^b versus K^{bm1}). This molecule will fail to present the foreign peptide requiring the missing subsite for binding. TCRs selected by the original self peptide–self MHC molecule combination will fail to interact with this new MHC molecule even when complexed with the appropriate foreign peptide, because of the TCR–MHC molecule mismatch (middle figure). These two events account for MHC restriction of T cells. But a TCR repertoire suitable for a response to this new foreign peptide–MHC combination would be selected in the individual expressing this particular MHC molecule as self.



R.N.G.

and considering the scale of peptide size versus the structure of the peptide-binding site, with its raised α -helical walls, intimate contact between the TCR and peptide probably necessitates simultaneous TCR-MHC interaction^{12,13}. Furthermore, there is indirect evidence that during antigen recognition TCRs do make specific MHC contacts that are influenced by, or directly involve, allelically polymorphic residues^{7,14-17}. To accomplish the optimal diversification of the peptide-binding site, in some cases alterations in regions simultaneously accessible to both the TCR and peptide could have been selected; as a result, during peptide recognition, TCR-MHC interactions occur that need to take this allele-specific structural variation into account. To achieve the selection of receptors that can meet this requirement, TCRs are 'pre-tested' on self peptides bound to the presenting molecules available. Selection for TCRs able to bind self peptide presented by a given MHC molecule will *pari passu* involve selection for adaptation to these allelically variable features of MHC molecules. This accommodation to self-MHC polymorphism does not need to contribute in a positive sense to the molecular interactions¹³. Rather, the receptor must prevent the unique allelic features of the self-MHC molecule from interfering with recognition of the bound peptide (self or foreign). This is an inversion of the usual thinking about thymic selection. Allelic polymorphism of MHC molecules is now seen to make its main contribution to selection by choosing peptides for presentation, and the principal binding event involves peptide recognition, not affinity for allele-specific MHC structure itself. In this view, T-cell bias towards self-MHC structure is merely a by-product of selection for recognition of self peptides bound by those MHC molecules. The self-peptides thus act as 'stand-ins' (structurally related analogues) for the unknown set of foreign peptides to be presented to mature T cells. MHC restriction would thus involve differences in the set of bound peptides that can be presented and the interfering effects of non-self MHC polymorphism on TCRs binding to peptide-MHC complexes.

Taking the crystallographic data² on the existence of peptide-binding subsites into account, this model suggests that the spatial relationship of the site of TCR-self-peptide contact (epitope) to the sites of TCR-MHC contact is constrained by the subsite into which the peptide binds. This leads to the selection of a set of TCRs that are accommodated to the MHC molecule in an orientation which, for effective recognition, requires that the epitopes of foreign peptides must be similarly located with respect to the overall MHC-binding site. This can be accom-

Quasicrystals turn to the sixth-dimension

LOOKING at the shadow of a thin slice of a simple cubic lattice you will see, depending on the lattice's orientation, a square or a hexagonal array. Or you may even see a seemingly disordered pattern of dots, although in fact along each of two directions, the lattice points will be arranged according to two incommensurate lattice spacings. Quasicrystals, such as discovered by Shechtman *et al.*¹ in 1984, exhibit a similar type of three-dimensional disorder, and can also have incommensurate lattice steps in the same direction.

from left to right across the picture. At one end, we look along an axis with fivefold symmetry to see an incommensurate quasilattice; at the other, we look along a four-fold direction to see a rational lattice. In between, the angle of view changes. The picture thus represents a kind of interface between quasicrystalline and truly crystalline phases and as such might be compared with actual micrographs of grain boundaries.

The authors have compared the picture to the well known *Metamorphosis* print by



By analogy with the cubic-lattice example, this suggests that quasicrystals can be regarded as three-dimensional projections or 'shadows' of a six-dimensional regular lattice. As with the cubic-lattice case, the projection can be rotated to give a different view². Indeed, the concept goes back at least to Coxeter's shadows of parallelotopes³.

Torres, Pastor and Jiménez, using algorithms developed to study quasicrystals^{4,5}, have drawn a composite view of a three-dimensional slice of a six-dimensional 'hypercubic' lattice, with the projection orientation varied continuously

M.C. Escher. It is a kind of Bloch wall, rotating with one parameter from one structure to another across a boundary of finite thickness.

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plished in most cases only by binding the foreign peptide to the same subsite, based on a structural similarity with the MHC-interacting portion of the self peptide. In the example reported by Nikolić-Zugić a change in or loss of a given binding pocket in the K^{bms} molecule presumably eliminates the presentation of a subset of self peptides whose epitopes guide the selection of certain TCRs, specifically those able to see the OVA peptide bound to this same subsite in the wild-type K^b molecule. The alteration of this binding pocket would also account for the failure of the

K^{bms} molecule to present the OVA peptide to OVA-K^b-specific T cells.

This model has a general and testable prediction — individual mutations of a given MHC molecule in TCR-inaccessible locations affecting the binding of distinct subsets of foreign peptides will have similar selective effects on the binding of self peptides. This in turn should lead to predictable effects on intrathymic positive selection of the repertoire for these same foreign peptides. The analysis of antigen responses by transgenic mice produced with such mutant MHC molecules pro-

vides an elegant, albeit cumbersome, method for testing this prediction. A more rapid, if less complete, approach would be to carry out the test *in vitro*; the ability to detect foreign peptide-independent, self-MHC recognition by antigen-specific T-cell clones and hybrids may provide the means to do so.

Although these new results and the model I have proposed address the molecular relationship between self- and foreign-peptide recognition by T cells, they do not resolve the central paradox of

thymic selection — the generation of a repertoire that is usable despite positive and negative selection events involving the same TCRs and MHC molecules. Many opinions have been expressed on this matter, but it remains the task of future experiments to unravel the paradox. □

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MOTION PERCEPTION

Switching off an after-effect

Oliver Braddick

COGNITIVE psychologists' accounts of visual information processing usually distinguish between 'pre-attentive' processes, which proceed independently of the subject's active control, and 'post-attentive' processes, whose operation is determined by what component of the visual input has been actively selected. Increasingly neurophysiologists have been looking for modulations of neural activity in awake animals which might reflect this selective control. Recent psychophysical experiments reported by Chaudhuri on page 60 of this issue¹ imply that the primary processing of visual motion information, which in most schemes would be placed firmly on the pre-attentive side of this divide, is in fact under the control of selective attention.

In the motion after-effect (MAE), prolonged viewing of a moving region induces a strong sensation of opposite motion in a stationary test pattern which is subsequently viewed. This is believed to reflect adaptation of the responses in directionally selective neurons, as has been demonstrated in the visual pathway of rabbits² and cats³. Chaudhuri shows that if subjects have to monitor a sequence of rapidly changing letters and numbers, the MAE produced by a surrounding field of moving spots is reduced in duration by almost 70 per cent, compared to tests when the character sequence is still present and fixated but requires no action.

The most obvious, and probably the most important, mechanism of visual selective attention is the control of foveal

fixation. But so much interest centres on the possibility of selective processes acting directly within the visual pathway, that the control of visual input by eye movements is generally regarded in this field as a disturbing experimental artefact. Chaudhuri recorded the subject's eye movements and showed that the attentional task did not change them enough to account for his results. More positively, attention could modify the MAE even when the subjects were required to attend to a location different from the position they were fixating.

The selective process demonstrated in these experiments is spatial; attention is devoted to one region of the visual field at the expense of another. It is also possible to select one attribute of a visual stimulus over another at the same location — for example to respond to colour rather than to shape or movement. Chaudhuri tested for such an effect by requiring his subjects to monitor the rapidly changing colour of the moving random-dot display, but this had no effect on the MAE. It is plausible that the neural basis of selection may be different for selecting a location and for selecting an attribute; the fact that visual areas are interconnected, topographical maps of the visual field is suggestive for possible mechanisms in the case of location. Clear analogues of spatial selection have been seen in the responses of single neurons^{4,5}; although modulations of the relative selectivity of single neurons for colour versus pattern have been reported⁶, they are not always clear cut⁷. But it is likely

that the colour and motion properties of the stimulus are processed by distinct cortical streams⁸, so selection between them may well reflect different processes from those operating within the representation of the visual field in a single cortical area.

The studies of attention modulating neural responses have concentrated on extrastriate cortical areas (V4, IT) in the temporal stream of processing. Little effect of attention has been demonstrated in the primary striate cortex⁹, and Chaudhuri uses this to suggest a post-striate contribution to the MAE. Primary directional selectivity arises both in striate cortex and (at least for higher velocities) in the post-striate area MT (ref. 10); so his idea is not unreasonable, although it is perhaps surprising that the post-striate contribution should be so large at the relatively low velocities tested. But the processing stream leading to MT includes its own specific population of striate cells and is separate from the temporal stream in which attentional effects have been studied, so the anatomy of attention in relation to motion is still almost completely speculative.

Whatever the anatomy, Chaudhuri's results show that selective attention can modulate a process which must be regarded as coming early in the sequence of processing visual information. Early effects of selection, though, should not be confused with the long-standing psychological issue of an early or late site for the processes that determine selection. Anatomically, it is clear that the flow of information in the visual pathway cannot be one-way¹¹, and a wide range of cortical structures may be able to modulate visual signals as early as the lateral geniculate body¹². This 'top-down' control means that there may be little truly 'pre-attentive' processing beyond the retina. But that does not excuse us from the need to understand essential distinctions between the ways different visual processes are subject to, and require, active control. □

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Glimpsing the hidden majority

Per Erik Ahlberg

WHAT are we to make of *Anomalocaris*, a metre-long fossil with two rows of fins at the back, a pair of arthropod-like claws at the front and mouthparts shaped like a pineapple ring? Or *Wiwaxia*, a rounded, headless organism covered in scales and spikes? These are just two members of a host of bizarre soft-bodied creatures known only from the Middle Cambrian Burgess Shale of British Columbia, a deep-water marine deposit in which numerous organisms were preserved because of anoxic conditions and near-instantaneous burial by mudflows. The presence of many otherwise unknown groups in the Burgess fauna underlines the biased and incomplete nature of the fossil record: fossils of the hard parts of animals can never by themselves yield a complete picture of early metazoan ecology or evolution. For this reason, the presentation at a recent meeting* of further fossils of soft-bodied animals from newly discovered Cambrian localities was the subject of considerable discussion.

The first hard-shelled animals appeared at the beginning of the Cambrian (about 560 million years ago). They evolved with explosive speed and by the end of the period, some 60 million years later, almost all the modern groups with mineralized skeletons had arrived. The emergence of these organisms was almost certainly accompanied by an even greater radiation of soft-bodied animals; it is sobering to realize that 85 per cent of the Burgess genera lack mineralized tissues¹ and would not normally have been fossilized. As the few shelly fossils — brachiopods, trilobites and hyoliths — are all common Middle Cambrian types, the fauna is probably a normal marine community, unusual only in being completely preserved.

Although most of the soft Burgess animals are strikingly different from the contemporary hard-shelled forms, the majority belong to familiar modern groups such as annelids, arthropods and priapulids. But some are so different from anything alive today that they defy classification. This problem is exacerbated by their isolated position in the fossil record. With no obvious close relatives to compare them with, it is hard to say which features of an animal such as *Anomalocaris* are restricted to that genus, and which are fundamental characteristics of the group to which it belongs.

The presence of relatives of both *Wiwaxia* and *Anomalocaris* in the new faunas, from the Buen formation of North Greenland and a new horizon in the

Burgess Shale itself, will help to answer such questions. The Buen assemblage is particularly important as it comes from the Lower Cambrian, close to the beginning of the metazoan radiation (S. Conway-Morris, University of Cambridge; J. Peel, Greenland Geological Survey). In general aspect it resembles the younger Burgess fauna: it is dominated by soft-bodied arthropods, some of which resemble the Burgess crustacean *Canadaspis*, but annelid and priapulid worms are also present. Trilobites and hyoliths are the only hard-shelled taxa. The preservation is good enough to show appendages and gut traces in many of the arthropods. Small shelly fossils of uncertain origin are common in Lower Cambrian rocks: one of these, the scale-like *Halkieria*, had been tentatively interpreted² as part of the armour of an animal like *Wiwaxia*. This hypothesis has been dramatically confirmed by the discovery in the Buen formation of a slug-shaped creature covered in a dense mail of *Halkieria* scales. In most respects it closely resembles *Wiwaxia*, although the armour is mineralized and slightly different in pattern, and the two can now be grouped together as 'halkieriids'. Their wider affinities are still in doubt, but they may lie with the molluscs³.

The original Burgess Shale locality lies on a steep mountainside and is part of a thick sedimentary sequence. Most of it represents deposition in deep water, but about 55 metres above the original quarry

GALACTIC STRUCTURE

Seeing the wood, not the trees

James Binney

WHAT shape is our Galaxy? We probably know much more about the form of neighbouring galaxies than we do about our own. Our view from half way out in the Galactic disk is highly skewed and horribly obscured by stars, gas and dust. Nevertheless, Leo Blitz (University of Maryland) and David Spergel (Princeton University) were able to reveal to a recent meeting* that the inner region of our Galaxy is highly elliptical, not symmetrical about its rotation axis as many had supposed. The problem now is to understand the Galactic dynamics that give rise to this form.

Blitz and Spergel draw their conclusion from a study of asymmetries in published surveys of emission in the 21-cm line of

shallow-water features start to occur; from this horizon comes a new and surprisingly different soft-bodied fauna (D. Collins, Royal Ontario Museum). Although it contains several now-familiar Burgess animals such as *Canadaspis* and the worm *Banffia*, it is dominated by a previously unknown arthropod with pincers and a spiky tail. There are also new representatives of more exotic groups, one of which is unmistakably related to *Anomalocaris*. The fossil shows the same pineapple ring array of sliding mouthparts and two rows of fins on the posterior part of the body, but the form of the dorsal surface and claws is different in the new form. As the new Burgess fauna is only slightly younger than the classic assemblage, the differences between them probably reflect ecological factors rather than evolutionary change. The new fossils seem to represent a shallow-water community, one that perhaps lived inshore of the typical Burgess animals.

The recent discoveries show the Burgess fauna to be just one of a widespread and fairly long-lived group of marine invertebrate communities. Our understanding of these soft-bodied faunas and their evolutionary significance⁴ is still incomplete, but more and more of the hidden majority of Cambrian animal life is now emerging into view. □

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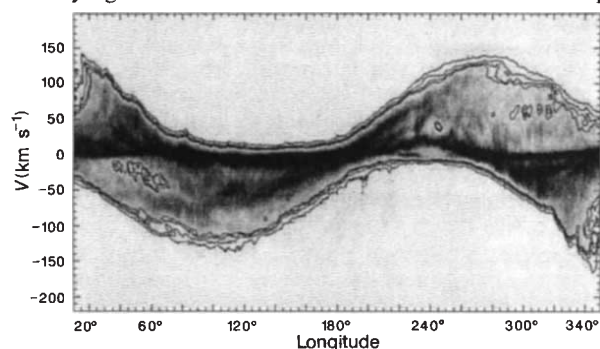
atomic hydrogen. When a radio telescope looks out from the Earth along a line that lies in the Galactic plane, it detects radiation from hydrogen atoms that are moving at up to 150 km s⁻¹ with respect to the Sun. The density of atoms in each velocity range depends on the angle l between the telescope's line of sight and the Galactic Centre (see figure, over).

The convex boundaries at upper right and lower left of the high-density region of the figure arise from gas at the outer boundary of the Galactic disk, about 18 kiloparsecs (kpc) from the Galactic centre (the Sun's galactocentric distance is $R_0 \approx 8$ kpc). If the Galaxy were axisymmetric, the velocity of the convex boundary at angle $l = 360 - x$ would be the same as that at $l = x$. Actually the two velocities differ by an amount $\Delta v(l)$ that falls from 20 km s⁻¹ at $l = 0^\circ$ to -20 km s⁻¹ at $l = 180^\circ$.

* The Palaeontological Association Annual Conference, Liverpool, 19–21 December, 1989.

* American Astronomical Society meeting, Washington DC, 9–13 January 1990.

Blitz and Spergel show that this difference can be nicely accounted for if the solar neighbourhood is moving out from the Galactic Centre at about 14 km s^{-1} . They argue that this outward movement arises because the solar neighbourhood moves around the Galaxy on an elliptical path and happens at the present epoch to be moving outwards. They show that a velocity profile $\Delta v(l)$ of the observed form would not arise if the Sun moved on a circular path and the outer galaxy were elliptical. This implies that if the Galaxy has a massive dark halo, this must be axisymmetric where it dominates the Galaxy's gravitational field.



Plot of the intensity of 21-cm radiation from atoms moving with speed V with respect to the Sun versus galactic longitude; zero longitude is the direction to the Galactic Centre.

The concave boundaries of the high-density region of the figure arise from gas closer to the centre than the Sun. The velocities of these boundaries at $l = 360 - x$ and $l = x$ differ by a smaller amount than the velocities of the concave boundaries. Blitz and Spergel conclude that hydrogen clouds interior to the solar orbit move on elliptical paths that are roughly similar.

What is responsible for the ellipticity of these streamlines? One possibility is spiral structure in the Galactic disk. But spiral arms are thought to be density waves encountered by stars in the disk as they orbit. Stellar populations with large random velocities are affected less by such waves than populations with small random velocities. Hence if spiral arms were responsible for the streamlines' ellipticity, the mean radial velocity of each stellar population would be correlated with its random velocity. Because no such correlation is found, spiral structure cannot be responsible and the culprit must be a massive non-axisymmetric component of some sort. Does this lie in the disk, or is the culprit one of the Galaxy's two thick components, the visible central bulge and the invisible dark halo?

About half of all spiral galaxies are 'barred spirals', in which a prominent bar links two spiral arms across the centre of the galaxy. Were our Galaxy of this type, the bar would rotate quite quickly and resonantly excite material at some radius between the Sun and the Galactic Centre. The similarity of streamlines at different radii argues against the existence of a

resonance, and therefore against the responsible component lying within the disk.

Because the demonstrated circularity of the dark halo at large radii makes it unlikely that this component is responsible for deforming streamlines into ellipses at $R < R_0$, Blitz and Spergel seek the culprit in an elliptical bulge. They find that, to fit the bill, the bulge has to be strongly elliptical and have a slowly rotating figure. At the solar radius, the bulge's figure needs to rotate at about 45 km s^{-1} . (To be compared with a circular speed of about 220 km s^{-1} .) Its axis ratio in the plane would be $b/a \approx 0.75$. Such a ratio within the plane is dynamically possible

only if the flattening of the bulge perpendicular to the plane lies near the upper limit of previous estimates.

Blitz and Spergel have assembled an impressive array of additional evidence to corroborate their picture. For example, they show that the model is compatible with the observed velocities of ionized and molecular gas away from the centre, and correctly predicts the velocity

of both gas known to lie at the very centre and an absorption-line feature which is seen against the Galactic Centre's continuum emission. The model also accounts for the small misalignment between the direction to the Galactic Centre and the ellipsoid formed by the random velocities of stars near the Sun.

Is the Milky Way unusual in having an elliptical bulge? We do not know much about the shapes of the bulges of external galaxies at radii comparable to R_0 . But from the work of Gerhard *et al.*¹ and Zaritsky and Lo² we do know that bulges are quite often triaxial near their centres, where they are readily observed.

One of the key issues in understanding how galaxies formed is whether the bulges of disk galaxies are qualitatively different from elliptical galaxies. Recent work³ strongly suggests that the great majority of elliptical galaxies are markedly triaxial. So Blitz and Spergel's conclusion that our own bulge is triaxial supports the conjecture that it is at heart a small elliptical galaxy. But if bulges and ellipticals are triaxial, why is the Galaxy's dark halo axisymmetric? □

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Inner tidiness

WHEN a woman is about to give birth, she frequently experiences a sudden compulsive urge to clean and tidy up. This surge of 'nest building' makes obvious evolutionary sense. But how is it triggered? Daedalus points out that many women experience a brief surge of the same instinct just before menstruation. Somewhere in the fluctuating skein of female hormones which control menstruation and pregnancy, he says, there must be a hormone that triggers the urge to clean and tidy.

So DREADCO's biochemists are taking blood samples from pregnant and premenstrual volunteers, and injecting selected chemical fractions into non-pregnant and post-menstrual volunteers. Those fractions which stimulate cleaning and tidying will be analysed further. Ultimately the compound responsible will be isolated in pure form. The DREADCO team will then establish its chemical structure and study its mode of action.

One crucial question is the effect it will have on men. The love of order is not a uniquely female virtue, so Daedalus hopes that his new hormone composition will trigger it in men too. A new and powerful psychotropic drug will have been born.

Like most hormones, DREADCO's Immaculone® will probably not survive in the stomach, and will be delivered by depot injection or slow-absorption skin patch. Housewives faced with spring cleaning, or scientists bemused by experimental chaos, may clamour for the treatment. But its major impact will be legal and social.

Immaculone will wonderfully reform vandals, hooligans, rioters and similar destructive criminals. One long-lasting depot injection should convert them to model citizens, given to picking up litter, queueing neatly at the bus stop, darning their socks, shaving carefully every morning, and, of course, turning up promptly on the appointed day for their next injection. The treatment would not curb criminality as such — a messy and destructive burglar on Immaculone would merely be converted into a neat and efficient one, much harder to catch. But crimes of vandalism, whose whole point is damage and disorganization, would be stopped in their tracks.

In a wider social context, Immaculone may have damaging side effects. Managers and politicians on the drug would certainly become more effective, but would probably resent the essential diversity and untidiness of life itself. They would enforce rigid, centralized, uniform, totally uncreative bureaucracies of the 'socialist state' variety. Daedalus is setting up a fake 'free medical check-up' clinic in Westminster, to measure the levels of Immaculone naturally present in the blood of our more committed advocates of left-wing revolution.

David Jones

Salinity threat to Upper Egypt

SIR—Salinization of land in arid regions has led to a decrease in the fertility of soils in many areas of the world. In Egypt, salinization poses a threat to agricultural productivity as well as to the survival of many antiquities, mostly in irrigated land.

Leaching of accumulated salts by annual inundations previously prevented land salinization in the Nile Valley, but that ceased when the Aswan High Dam was completed in 1965^{1,2}. By 1977, new salt deposits had been observed at the foundations of ancient monuments, but the reasons for the increase of groundwater salinity have been far from clear.

We selected the Karnak/Luxor region for our studies because it is representative of archeological sites in Upper Egypt. It includes the Karnak Temple, one of Egypt's most important archaeological monuments, located between irrigated fields and the Nile River^{1,2}.

Samples of groundwaters from the headquarters of the Canadian Archaeological Expedition immediately east of Karnak and of Nile water near Karnak were collected in 1977 and 1987, were analysed to determine Na⁺ and K⁺ concentrations using atomic absorption spectroscopy and were then compared with data assembled earlier^{3,4}. It was determined that the concentrations of these ions in the Nile fluctuate within merely $\pm 25\%$ over a period of eighteen years (see table). We have taken these ions as a proxy for salinity for that reason, because they are the most abundant ions in groundwater and because they are expected to be relatively unreactive with the soil. As an index of groundwater salinity, we took the ratio of the Na⁺+K⁺ concentration in the groundwater sample to the average concentration of these ions in the Nile River at Karnak. If these high concentrations of Na⁺+K⁺ in 1969 were due to the previous inundation regime, they would have been leached out between 1969 and 1987. That there has been no such effect is not surprising: irrigation in Egypt has been stable for millennia.

Alternatively, high groundwater salinity could have arisen by the diffusion of salts in soils in the nearby desert, previously unaffected by irrigation, into agricultural areas. If such diffusion had been significant, a time-dependent concentration gradient would have been generated, but none has been observed.

The only remaining process that could account for the high salinity of the groundwater is evapotranspiration. If that were the only factor responsible for the observed groundwater salinity, the implication would be that $89 \pm 3\%$ of the water

	Groundwater	Nile water	Concentration ratio
1969–70	9 $\pm$ 2*	1.1 $\pm$ 0.2	8 $\pm$ 2
1977	10.6 $\pm$ 0.2	1.52 $\pm$ 0.03	9 $\pm$ 2
1987	10.2 $\pm$ 0.7	0.99 $\pm$ 0.03	9 $\pm$ 2
Average	10.0 $\pm$ 0.7	1.2 $\pm$ 0.3	9 $\pm$ 1

* Average of nine wells collected throughout Karnak^{3,4}.

now used for irrigation evapotranspires at the ground surface of the cultivated fields. This result is in itself remarkable. In the Colorado River Basin, for example, approximately only 75% of the water applied to irrigated fields is lost to evapotranspiration⁵. The climate of Upper

Egypt, situated between the Sahara and the Eastern deserts, is hotter and drier than in the Colorado Basin.

It therefore appears that evapotranspiration is an important process significantly contributing to the salinity of the groundwater in the Karnak area. Since 96% of the groundwater, which flows from the irrigated fields into the unirrigated land where the Karnak Temple Complex is located, evaporates under the temples², practically all of the salts originally present in the irrigation waters are being deposited at the ground surface and, more devastatingly, in the foundations of the monuments at Karnak. Because irrigated fields are located along the Nile River over a large part of Egypt, we expect that the Nile Valley south of Fayum will be affected much as we have found the Karnak region to be.

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Leaky answer to greenhouse gas?

SIR—The British government is encouragingly positive towards action to reduce global warming from an increasing 'greenhouse' effect, using in particular arguments endorsed by the House of Commons Energy Committee¹, that favour electricity generation from gas rather than coal. The case is based on the relative carbon coefficient of various fuels, essentially a measure of CO₂ emission per unit of energy, and which are 0.75 for coal, 0.62 for oil and 0.43 for gas². But does that argument hold up?

Methane makes up 75% of natural gas, has a stronger greenhouse effect than CO₂ and its concentration in the atmosphere is increasing faster³. Because methane (CH₄) blocks radiation of wavelengths at which the atmosphere is relatively transparent, its 'greenhouse' strength per molecule is about 25 times stronger than that of CO₂ (ref. 1). Releases of methane associated with coal mining and gas production may be significant^{4,5} relative to the biological production. The present increase of 1% per year (several times that for CO₂) may reflect decreased OH density due to pollutants such as CO, but in part reflects the increasing release rates.

The production and delivery of natural gas necessarily involves leakage — at the sea well-heads and high-pressure pumps (to 1,500 p.s.i.), at the shore hand-over and booster station, and in the local distribution net and beyond the meter. Gas leakage in the local net can be judged on the basis of the metered difference attributed as 'unaccounted for'. Through

their recent substantial pipe-replacement works, British Gas has cut this from values that were as high as 15–20%. The present 'unaccounted for' rate is 3–4% in Wales, little different from the UK average (J. Burrows, personal communication). British Gas told the Energy Committee¹ that methane leakages from the gas grid amount to 400,000 tonnes per year, nearly 1% of throughput, but this is a politically motivated lower limit. Household gas meters are accurate to $\pm 2\%$ so I will assume 2–5% as a realistic range for leakage from the low-pressure delivery net. Leakage rates for sea-based production are unavailable, but I estimate it to be 1–5%, based on information from the Piper Alpha disaster inquiry. This estimate of 3–10% total losses is similar to the 3–6% derived for land-produced natural gas in the United States⁶, where there are legal restrictions and accounting for leakage.

Hence the relative greenhouse effects, correcting for the 3–10% leakage and expressed per carbon atom, are $G_{\text{gas}}:G_{\text{coal}} = [0.43 + \eta 0.75 (0.03 \text{ to } 0.1) \times 25] : 0.75 = 0.57 + (0.75 \text{ to } 2.5) \eta$, where η is the ratio of atmospheric residence times for CH₄ and CO₂. Evidently the value of η is significant. The effective residence time for CO₂ is uncertain, whereas that for the light CH₄ is the stratospheric lifetime of 6.5 years (ref. 4) augmented by the upward diffusion time to a total of about 10 years. CO₂ interchanges with oceans and biomass, a substantial fraction being 'fixed' as the annual atmospheric loading is only 50% of the global fossil-fuel burn. The

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effective residence time probably exceeds the 7-year interchange time^{7,8} possibly being as high as 20 years. Thus the relative greenhouse effects are $G_{\text{gas}}:G_{\text{coal}} = 0.95$ to 4.1. This calculation neglects the contributions of ethane, some 20% of North Sea gas, that need specific study. Also, from deep-mined coal in the UK, roughly $20 \text{ m}^3 \text{ t}^{-1}$ of methane is released, discounting that drained off and burned. Including this average figure, the greenhouse factor from coal is increased to $G_{\text{coal}} = 0.75 + 0.21\eta$

and the relative effect to $G_{\text{gas}}:G_{\text{coal}} = 0.8$ to 3.0.

Although the uncertainty is large, the substitution of gas-fired stations for coal is likely to worsen the greenhouse effect. If its case to the European Commission is to hold up, the Government needs to look much more carefully at the gas leakage problems.

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Protease or protease inhibitor?

SIR—Richardson *et al.* have proposed¹ that the intensely sweet protein thaumatin may act as a protease inhibitor on the basis of its homology with the maize bifunctional inhibitor. We wish to point out that thaumatin also has a significant homology with a group of recently identified picor-

the cysteine residue is freed from its disulphide bridge by dithiothreitol and made available as a nucleophile in proteolytic cleavage.

Also shown in the figure are the corresponding regions of the maize bifunctional inhibitor and a tobacco protein induced on

TRYPSIN	L E G G K D S C Q G D S G G P V V C N G Q L Q G I V S
HRV2 2A	L I G E P P C E P G D C G K L L C K H G V I G I V T
THAUMATIN I	D S G S G I C K T G D C G G L L R C K R F R P P T T
MAIZE 22K INHIB	A S R G S C R T G D C G G V V Q C T G Y G R A P N T
TPR	G S G R G N C E T G D C N G M L E C Q G Y G K P P N T

Alignments of the sequences around the active site nucleophile of trypsin and the 2A protease of HRV2 with sequences from thaumatin I, the maize 22 K bifunctional inhibitor and the tobacco PR protein. Residue numbers: porcine trypsin, 174-200; 2A protease, HRV2, 95-121; *thaumatococcus daniellii* Benth, 52-78; maize 22 K bifunctional inhibitor, 55-81; pathogenesis-related protein induced in tobacco by infection with TMV (TPR), 54-80. Similar residues are boxed.

naviral proteases² and that this may explain the known protease activity of thaumatin³.

The sequence surrounding the active site of the 2A protease of human rhinovirus 2 (HRV2) (see figure) was used to search the PIR and Swissprot protein data banks using the FASTP program⁴. Similarity was found with trypsin and other serine proteases², but also, more surprisingly, with thaumatin I (Z values; thaumatin I, 5.8; trypsin, 5.6). In particular, the sequence around the nucleophilic active site, -GDCGG- is present in thaumatin I; all other boxed residues, with the exception of the second glycine, are typically found near the active sites of serine proteases.

We propose that the cysteine residue in the -GDCGG- sequence may be the active site of the thaumatin protease even though the crystal structure of the molecule shows that all 16 cysteines are occupied in disulphide bridges⁵. The protease activity of thaumatin is observed only upon addition of dithiothreitol³. As the -GDCGG- sequence is exposed on the surface of the thaumatin molecule, it is conceivable that

TMV infection (TPR). The similarity of the two proteins to thaumatin has prompted the suggestion that thaumatin may function as a protease inhibitor^{1,6}, but both proteins also have a significant similarity with the active site of HRV2 protease 2A. As the TPR protein has not yet been shown to act as a protease inhibitor (it does not, for example, inhibit trypsin), it may be of interest to examine

whether it possesses a protease activity instead.

The finding of significant similarity between thaumatin and two classes of proteins, one of proteases and one of protease inhibitors, emphasizes the care with which the results of computer searches must be treated.

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The aroma of rice . . . and tiger

SIR—About 8 per cent of the active component of the aroma of fragrant varieties of Indian-Basmati rice has been identified as 2-acetyl-1-pyrroline¹. But it may be of interest that other components of rice aroma are likely to include a constituent of the volatile fraction of the 'marking fluid' of the tiger, believed to be a source of pheromone in this animal². We have observed the distribution pattern of 9,662 markings of 10 tigers in the open-air zoo of Nandan Kanan; the results suggest that the fluid encodes territorial and sexual connotations.

Of the volatile molecules of marking fluid we have recently studied the most elusive is one with a pleasant aroma. In its hydrochloride form, this aroma is not perceptible but the molecule can be separated from other amines such as putrescine, cadaverine and phenylethylamine by paper chromatography (solvent, *n*-butanol:acetic acid: water-4:1:1), and compared with the active component in the aroma of rice (cooked or uncooked) also in its hydrochloride form. In co-chromatograms, rice aroma and tiger aroma have the same R_F , established by cutting out the pieces of the chromatogram in an ordered sequence and moistening them with alkali. This similarity was confirmed independently. In the same chromatogram, synthetic 2-acetyl-1-pyrroline (see ref. 3) had a far higher R_F value. As habitual rice-eaters, we can detect a difference between these two aroma.

Volatiles of tiger and rice aroma also share at least one peak, as is evident from gas-liquid chromatography (with a squalane and Carbowax 20M packed column). In a tiger cub we reared, the tiger aroma was absent at an early age, but appeared later (after the aroma had become perceptible to us). Attempts to further characterize the volatile fraction (using gas chromatography and mass spectrometry) failed, possibly because by the time samples reached the analysis centres at Bangalore (India) and Swansea (UK), the minute quantities of this rather unstable substance had vanished.

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Gems from the archive

W. F. Bynum

Establishing the New Science: The Experience of the Early Royal Society. By Michael Hunter. *Boydell & Brewer, Woodbridge, Suffolk IP12 3DF, UK: 1989. Pp.382. £45, \$86.*

Science in Sweden: The Royal Swedish Academy of Sciences 1739–1989. Edited by Tore Frängsmyr. *Science History Publications, Box 493, Canton, Massachusetts 02554, USA: 1989. Pp.291. North America \$45, elsewhere \$49.50.*

WHETHER scientists as individuals are naturally more gregarious than other people may be debated, but modern science is inextricably associated with the scientific society. Science today seems inseparable from the plethora of organizations which in turn count the holding of meetings as one prime function. Communication is a central part of scientific activity.

These two books explore aspects of two venerable scientific societies, the Royal Society of London and the Royal Swedish Academy of Sciences. The collection of essays edited by Tore Frängsmyr appeared in time for the two hundred and fiftieth anniversary celebrations of an organization most famously identified with the selection of the recipients of the Nobel prizes for chemistry and physics. Michael Hunter's volume concentrates on about four decades in the history of the institution with good claims to being the original mod-

ern scientific society. Indeed, had Linnaeus, a founder member of the Royal Swedish Academy of Sciences, been given to classifying organizations as well as organisms, the Royal Society of London could have provided him with the holotype for *Societas scientifica*. Since then, many permanent varieties have been formed.

Hunter's reputation as one of the foremost students of Restoration science in England can only be further enhanced by this volume. The book admirably displays what are always characteristics of Hunter's scholarship: a mastery of printed primary and secondary sources combined with a sharp eye for new archival gems. So much has already been written on the early history of the Royal Society (beginning with Thomas Sprat's monograph as early as 1667) that non-specialists might

be forgiven for assuming that there is not much more to say. On the contrary, most of Hunter's chapters are based on previously unknown manuscripts, some of

them in the archives of the society itself.

This is not a continuous narrative history of the society but a collection of individual, loosely connected essays (about half of which have already appeared in journals or other volumes, and one of which is written jointly with Paul Wood). Some of them concentrate on individuals (Sprat, Henry Oldenburg, Robert Hooke), others on more collective aspects of the society's activities — its finances, committee structure, and attempts to establish research and educational facilities and to find paid employment for more of its fellows. By no means all the case studies Hunter recounts had satisfactory outcomes, but a modern scientific community which sometimes feels itself beleaguered and unappreciated will find much of resonance in Hunter's book.

Take the issue of finance. Charles II graciously allowed the new society to advertise its royal patronage, but there was not much forthcoming from the royal exchequer. From the beginning, money to run the society depended largely on subscriptions from its fellows and from bequests from well-disposed patrons. This in itself made recruitment a key issue, but by the late 1660s, when the first flush of enthusiasm began to fade, fellows began defaulting on their annual subscriptions and attendance at meetings fell off. Early on, a series of committees had been established in such diverse fields as mechanics, agriculture and the "history of trades", in an effort to provide foci for the diverse

IMAGE
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interests of members. This "experiment in corporate enterprise" had not succeeded and, by 1673, Seth Ward pronounced the society to be in a state of crisis.

Shaky finances made it difficult to meet the costs of demonstrations at the society's meetings, the burden of which often fell on Robert Hooke, the society's paid "curator". Hooke also received (or was supposed to receive) an annual stipend from Sir John Cutler, a wealthy businessman and philanthropist, in return for an annual series of lectures. Hunter's fascinating analysis of Hooke's attempt to recover through a lawsuit the arrears of Cutler's bequest is a salutary tale of the conflict between patronage and scientific freedom.

Finances were also central to the society's unsuccessful attempt to transform its "cabinet of curiosities" into a full-scale

museum and research collection, or its abortive plan to acquire, in 1667–68, its own headquarters and to establish there a “philosophical college”. A site was found, Christopher Wren designed a building and money was pledged. But the money promised was not enough, and the society had to wait until 1710 before it acquired its own premises.

It is well known that British science remained predominantly ‘amateur’ for more than two centuries after the foundation of the Royal Society. Hunter’s volume demonstrates that a core of early fellows — Hooke, Boyle, Grew, Oldenburg — recognized that science needed more cultivators than merely those whose financial or social circumstances gave them leisure. They wanted election to the society to require more than casual interest and the means to pay an annual subscription. Many of the episodes recounted here have been neglected by historians because they led nowhere, but they reveal much about the values of those who created the society and help us to understand why the subsequent pursuit of science in England took the shape it did.

By contrast, the Royal Swedish Academy had the potential of other, continental role models, especially in the area of salaries for members which those in the French Académie des Sciences enjoyed. In fact, the Swedes adopted the English prototype, though the academy had official responsibilities in areas such as examining patent applications and an external income through the exclusive right — granted in 1747 — to publish the Swedish almanac. The academy lost this monopoly only in 1972 and with print runs of a staggering 294,000 as early as 1785, the profits were considerable. The ten essayists whom Frängsmyr has gathered recount a variety of the academy’s principal activities, from funding scientific travel and exploration to running the Swedish Museum of Natural History and an astronomical observatory; from involvement in environmental protection research and campaigns to research institutes in mathematics, marine biology and astrophysics. Nor is the impact of the Nobel Foundation on the academy’s fortunes and international standings neglected.

As is perhaps appropriate for a commemorative volume such as this, the authors write with affection for the institution they are describing. Not even they would argue that academicians could quite claim “*la science en Suède, c’est nous*”. But so wide-ranging have the academy’s activities been that the book’s title seems entirely appropriate. □

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Learning through practice

John A. Campbell

Computers and Thought: A Practical Introduction to Artificial Intelligence. By Mike Sharples, David Hogg, Chris Hutchison, Steve Torrance and David Young. MIT Press: 1989. Pp.401. Hbk \$25, £22.50; pbk £13.95.

ARTIFICIAL intelligence (AI) is a subject now well served for introductory texts at various levels. But there is still a noticeable gap between popular accounts and presentations of basic AI techniques for an audience of specialists in computing. This is a gap that the authors of *Computers and Thought* aim to fill. Their explicit goal is to “introduce people with little or no computing background to AI and cognitive science”. Their material has been developed from a regular ten-week course given to first-year arts undergraduates.

Knowledge representation and search are the two main pegs on which the book hangs a respectable amount of information about both the scientific perspective and the computing techniques of AI. In my biased opinion, this is the right choice of design. Readers of *Nature* who attempt the course should be able to learn what the simplest relevant techniques are and how to exploit them, because of the examples of programs that are presented. Most of these examples gain in coherence because they are solutions for parts of one problem: construction of a program to give tourists rather basic advice about entertainment and transport in central London.

This coherence may escape some of the original intended customers. The programming language used is POP-11, which need not frighten anyone with past exposure to Pascal or C. But it has been observed in more than one country that the most common reaction produced in arts students by even small chunks of Pascal is terror. It would have been interesting if the authors had revealed how they have managed to protect their own students from this effect.

Readers who have no difficulty with the conceptual details of the programming examples may have two other difficulties with the presentation. The first is at the nuts-and-bolts level: POP-11 arcana like the differences between x , $?x$ and $??x$ are inclined to turn up without real explanation. The second is more important. It is that, although the scope and ambitions of AI are described well in various parts of the text, and although the programming examples have to fall far short of those targets if the book is to do a good introduc-

tory job for non-specialists, there is no significant attempt to put a bridge in place between these two levels.

There is thus a risk that a reader who wants to know about the differences between introductory exercises and what it feels like to be on the research frontier of AI will finally ask: “is that all there is to AI in practice?”. In isolated cases a proper answer is “yes”; in the others, it would have been helpful to have brief accounts of what the largest or most adventurous AI projects have needed, beyond just the basic techniques, to be successful. In an admittedly limited experiment, one scientist and two out of three arts graduates had varieties of this impression, unprompted, after looking at the book. (The third arts graduate was ambushed by ??s, despite being warned to stay out of the end-of-chapter appendices where most of the programs were lurking, and failed to stay the distance.)

People who are primarily looking for AI techniques and how to use some of them in programming will have no such problems, and will benefit from the clear explanations (in English rather than POP-11) that are given here. Because of the authors’ own research interests, the book contains examples for which basic discussion is hard to find elsewhere: natural-language processing and vision, for example. A rewarding further example is the treatment of specific models of cognition — especially of erroneous cognition, for arithmetic and learning of tenses of verbs — as sets of rules that can be used in programs.

This last example, and an excellent chapter on the immediate philosophical issues raised by AI, may whet the reader’s appetite to learn more about cognitive science. Appetites for this and other AI-related learning are provided for, at the end of the book, by an annotated list of further reading.

A diskette containing full programs for examples developed in the book is available separately, at a price in the United Kingdom of £14.25 (plus VAT). This should not be bought without careful inspection. On the copy that I was sent for review, about half the programs did not operate because auxiliary software that should have been present on the diskette could not be found when loading and compilation were attempted. Those programs that did run were time-limited, so that a user could work with them only for 10 minutes per session. This produced either undignified scrambles or sullen frustration, depending on the user’s degree of computer literacy. As one of the arts volunteer testers said, “this is no way to run a business”. □

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A gentleman's pursuit

Daniel J. Kevles

Reflections on Intelligence. By R. V. Jones. Heinemann: 1989. Pp.376. £19.50.

In *Most Secret War**, R. V. Jones provided a gripping memoir of British scientific intelligence from 1939 to 1945, when he was the head of scientific intelligence on Britain's Air Staff and scientific advisor to the British Secret Intelligence Service, or MI6. In *Reflections on Intelligence*, Jones reviews his postwar experiences in intelligence and offers some postscripts to the first volume, including a number of fascinating responses to it from participants in the secret war on both sides of the Channel. He attempts briefly — but appropriately — to show that systematic scientific support for the armed services began with World War I, and gives a much-enlarged, thoroughly absorbing account of the crucial Oslo Report (about which more below).

What makes the new book particularly inviting is Jones's concern to elaborate on some of the matters explored in the previous work, particularly to illuminate the craft and ethics of the security trade. To this end, drawing material from a broad range of historical episodes, he supplies thoughtful chapters on subjects such as official secrecy as well as on intelligence in relation to security, deception and command. He also offers apothegms of right and proper practice — for example, no agent should be intentionally betrayed; the gathering of information should use minimum force and trespass; excessive secrecy is counterproductive and sometimes silly; no matter how sophisticated technology may become, human spies will always be needed.

In a sense, the practice of intelligence violates the norms of conventional upright conduct ("gentlemen do not read each other's mail", the US Secretary of State Henry L. Stimson said, in 1929, when he shut down his country's cryptographic centre). Jones has his own views about the role of gentlemen in the intelligence game. His sensibilities express themselves throughout the book but nowhere more clearly than in his extended treatment of the Oslo Report.

The report came into Jones's possession in November 1939, having been recently received at the British Legation in Oslo, Norway. It described in seemingly authoritative detail several important German technical developments, including rocket activities at Peenmünde and a radar system for the guidance of German

bombers. An item of independently obtained intelligence persuaded Jones that the Oslo Report was not a plant sent to mislead but a genuine and revealing account of certain key military technologies. In a review of scientific intelligence that he wrote in April 1946, Jones called the document "one of the most brilliant intelligence reports received throughout the war", adding, however, that the source of the report had not been discovered.

Jones discussed the Oslo Report in *Most Secret War* but did not reveal the full text for fear that it might contain a clue to

to Norway, Mayer pecked out the Oslo Report on an old hotel typewriter, expecting at first to send it to Turner but then concluding that it would be safer delivered to the British Legation. In August 1943, Mayer was arrested by the Gestapo for listening to BBC broadcasts. He spent the rest of the war in various concentration camps and, after a few postwar years on the Cornell University faculty, returned to Germany, where he resumed his career, neither recognized nor hounded for his pro-Allied espionage.

In recent years, a number of revelations about the game of intelligence have

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R. V. Jones — intelligence officer and author, he was in charge of British intelligence against the V-1 (flying bomb) and V-2 rocket (right), and played an important and successful part in British Scientific Intelligence during the Second World War.

the author's identity — which by that time Jones knew — and might thus, perhaps, lead to his embarrassment. The text has since been published in volume I of the official *British Intelligence in the Second World War*, and the author, dead for several years, is beyond embarrassment. Jones now freely relates his discovery, in the mid-1950s, of who wrote the report — an absorbing adventure that turned on several chance encounters, one involving a toy pneumatic rubber monkey, that led eventually to Hans Ferdinand Mayer, a physicist who before and during the war had been head of the central laboratories of Siemens & Halske, the noted electrical company.

Although Mayer had studied with Philipp Lenard, a staunch enemy of Einstein's "Jewish physics", he detested the Nazis and admired the English, especially an English businessman named Cobden Turner, with whom he had dealt for many years. Mayer had told Turner about a half-Jewish girl who had been repudiated by her Nazi father and could not leave the country with her mother; Turner arranged for the girl's emigration to England and installed her as a member of his household. While on a business trip

suggested that it is dirty and devious, a trend that Jones deplores. He contends that intelligence benefits from abiding by the rules of gentlemanly conduct, noting, for example, that Niels Bohr was "happy to co-operate with the British Secret Service because he found that it was run by a gentleman" (p.332). Jones judges the morality of Mayer's pro-Allied treachery as superior to that of Kim Philby's pro-Communist perfidy. He sees Philby's as distasteful and cold-blooded, even if idealistic; Mayer's as the result of devotion to the long-term interests of the great European family and to the decencies of civilization that he found embodied in Cobden Turner. Defending those decencies may now seem antique, and identifying them with gentlemen may seem somewhat snobbish, yet there is a good deal to be said for the code of values that people like R. V. Jones brought to the practice of intelligence, which, once integrated into the protection of national security, can all too easily value nothing higher than its own success. □

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*Published as *The Wizard War* in the United States.

Endless forms most beautiful

Jerry A. Coyne

Genetics, Speciation and the Founder Principle. Edited by L. V. Giddings, K. Y. Keneshiro and W. W. Anderson. Oxford University Press: 1989. Pp.373. £54, \$65.

ALTHOUGH Darwin's finches hold pride of place as examples of rampant island speciation, they are far overshadowed by the *Drosophila* of Hawaii. Only one ten-thousandth of the Earth's land surface, the Hawaiian islands harbour more than a quarter of its 2,000 species of *Drosophila*. The systematics and genetics of this group, moreover, are far better understood than those of any other island radiation.

The Hawaiian *Drosophila* story is due largely to one tireless man, Hampton Carson. After a distinguished career in the mainland United States, Carson moved to Hawaii and soon realized the value of the native flies for studying speciation. Following the taxonomic groundwork of Elmo Hardy, Carson assumed leadership of the Hawaiian *Drosophila* project, which produced massive amounts of ecological, genetic and systematic data. This impressive body of work is commemorated in Carson's Festschrift, *Genetics, Speciation and the Founder Principle*, devoted mainly to *Drosophila* but also describing work on plants, mole rats and the Yanomama Indians. The research ranges from old-fashioned natural history to DNA sequencing, its variety a tribute to Carson's broad approach to speciation.

The book is, however, more than just a Festschrift: it is a manifesto for the Hawaiian group's theory of speciation, the 'founder effect'. The older explanation for riotous speciation on islands and archipelagos is that suggested by Darwin and elaborated by his successors: these areas provide a unique combination of empty habitats, novel environments and geographical isolation. Rare invading groups evolve quickly, producing species as a by-product. As suggested by its name, 'adaptive radiation', this process is driven largely by natural selection, although sexual selection must often be important.

The founder effect theory, on the other hand, assigns a major role in speciation to genetic drift. According to this scheme, small colonizing populations experience random changes in allele frequency. This drift makes them temporarily maladapted, traversing valleys of low fitness until natural selection hauls them up a new adaptive peak. The process is similar to Sewall Wright's famous 'shifting-balance' theory of evolution, with the adaptive valleys representing reproductive isolation between species. Since first suggested by Ernest Mayr, the concept of the founder effect has undergone many changes, which Will Provine clarifies in a

fine essay. It is important, however, to distinguish this theory from that of founder events, the idea that speciation may occur after a few individuals invade a new area. Unlike founder effects, founder events are not disputed.

Understanding speciation on islands is a major task of evolutionary biology. Although this volume contains much fascinating information (particularly the work on Hawaiian plants by Ganders and Carr *et al.*), it does little to resolve the issue, because most authors do not weigh the founder-effect theory against its alternative. Instead, chapter after chapter describes intriguing work, but then argues *post facto* that the results are consistent with founder effects. Unfortunately, virtually every observation is also consistent with adaptive radiation. Epistasis between genes causing speciation, for example, is repeatedly cited as evidence for founder effects. Yet as Dobzhansky and Muller pointed out half a century ago, the neodarwinian model predicts similar results (genes causing hybrid sterility and inviability must do so by interacting with other genes). Rapid speciation after founder events, another favourite argument for founder effects, is just as consistent with the notion that strong natural selection follows infrequent colonization.

I am not sure why these authors reject the traditional view, and it is difficult to imagine what evidence would critically support their hypothesis. It is hard enough to demonstrate that a species was once small enough to experience strong genetic drift (and we have no such evidence in the Hawaiian *Drosophila*, which show much molecular polymorphism), but it is nearly impossible to show that this drift was an important cause of reproductive isolation. For exactly the same reasons, Wright's shifting-balance theory remains, after 60 years, a magnificent but largely untestable view of evolution in nature. There are, to be sure, a few laboratory experiments showing that repeated bouts of genetic drift can cause some reproductive isolation (two are described in this volume), but their design makes them rather unrealistic models of island speciation.

To be fair, it is nearly as difficult to find firm evidence for the process of adaptive radiation. Can we then draw any conclusion about how speciation works on islands? I think there are several reasons for favouring the traditional explanation. First, the theory of founder effects is not a model, but a set of verbal assertions. More

rigorous mathematical models by Barton, Rouhani, and Charlesworth (barely mentioned here) show that founder events very rarely produce founder effects. It therefore seems unlikely that more than a small fraction of island species can arise by that process. Second, the theory of founder effects invokes many more assumptions than that of adaptive radiation. The former requires complicated mixtures of special genetic architectures, repeated population bottlenecks, and strong epistasis, whereas the latter requires only that selected genes pleiotropically cause reproductive isolation (this is inevitable under sexual selection). Adaptive radiation is therefore likely to occur under a much wider range of conditions in nature. Third, there is no evidence that the genome must be disorganized by genetic drift before a new species can arise. Artificial selection on plants and animals readily produces aspects of reproductive isolation such as mating discrimination. Fourth, there are many cases of continental radiations in which speciation apparently followed the sundering of continuous habitat. These examples, which include Death Valley pupfish, Australian eucalyptus and neotropical butterflies, show that prolific speciation does not require founder events. Fifth, both continental and island species show recurring patterns of speciation: the preferential sterility and inviability of heterogametic hybrids (Haldane's rule), and its causation by genes on the X chromosome. These common patterns imply a common process in all species, not a unique evolutionary path for those on islands. Finally, there is evidence from Darwin's finches and the monkey flower *Mimulus* that reproductive isolation can result directly from adaptation in nature. We have no similar evidence for founder effects.

As a historical phenomenon, speciation is slow to yield its secrets. But its study is made even more difficult by a tendency to harmonize every observation with some favoured theory while ignoring the alternatives. The advocates of founder effects must tell us what observations can prove their theory wrong. Only then can we test it against its competitors and continue to build the remarkable edifice begun by Carson. □

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New In paperback

■ *What Do You Care What Other People Think?* by R. P. Feynman is his second and final volume of memoirs (see review *Nature* 336, 432; 1988). Published by Unwin Hyman, price £4.50.

■ *Great Geological Controversies 2nd edn* by A. Hallam is available from Oxford University Press, price £12.50. For review see *Nature* 306, 128; 1983.

The subducted component in island arc lavas: constraints from Be isotopes and B–Be systematics

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Although high concentrations of beryllium-10 and boron are taken as unequivocal indicators of the contribution of subducting plates, controversy persists about the processes by which material is transferred from slabs to the sources of arc magmas. Data on $^{10}\text{Be}/\text{Be}$ and B/Be ratios from four arcs suggest that the contribution from the slab is compositionally homogeneous in each arc and that subducted boron is not stored in the sub-arc mantle. The link between subduction and magmatism at convergent margins seems to be well regulated.

BORON and ^{10}Be are excellent tracers of sediment recycling at convergent margins. Both are strongly enriched in many volcanic arc lavas relative to mid-ocean-ridge basalts (MORBs), ocean-island basalts (OIBs) and reservoirs in the upper plate. Because the budget of these elements in arc magmas is dominated by the subducted component (ocean sediments, altered crust, or melts/fluid derived therefrom), the Be–B systematics of arc lavas are insensitive to chemical variation in the mantle and are largely unaffected by involvement of continental crust. They can thus be used to identify and ultimately quantify the subducted component in convergent margin magmas, and to more broadly assess subduction recycling.

^{10}Be is a cosmogenic and radioactive (half-life 1.5 Myr) nuclide, formed by spallation of oxygen and nitrogen in the atmosphere^{1–3}, which rapidly becomes concentrated in clay-rich ocean sediments. Thus, ^{10}Be concentrations are high in uppermost ocean sediments, averaging $\sim 5,000 \times 10^6 \text{ atom g}^{-1}$. The ratio of the cosmogenic isotope ^{10}Be to the stable isotope ^9Be in sediments is also high, with an atom ratio averaging $\sim 5,000 \times 10^{-11}$ (refs 4–6). By contrast, ^{10}Be concentrations in mantle-derived magmas from mid-ocean ridges, ocean islands and active continental rifts are generally below detection limits ($< 1 \times 10^6 \text{ atom g}^{-1}$) with a $^{10}\text{Be}/\text{Be}$ atom ratio of $< 5 \times 10^{-11}$ (refs 7, 8). Because of its short half-life, the high ^{10}Be concentrations ($1\text{--}24 \times 10^6 \text{ atom g}^{-1}$; refs 7, 9) and the high $^{10}\text{Be}/^9\text{Be}$ ratios ($1\text{--}81 \times 10^{-11}$; refs 8–10) measured in many arc lavas require recent incorporation of a component derived from young sediments⁷. Subduction processes taking longer, or involving sediments older, than five or six half-lives of ^{10}Be (7.5–9 Myr) will be undetectable. A combined study of beryllium isotope compositions and stable ^9Be abundances¹⁰ of the four arcs studied here indicated that the subducted component in arcs might be approximately homogeneous and was used to investigate two-stage models for subduction-zone mixing.

Boron is in many respects analogous to ^{10}Be . It is also strongly enriched in ocean sediments, reflecting its high concentration in sea water (4.6 p.p.m.) and its strongly adsorptive behaviour during sedimentation^{11,12}. Boron concentrations in sediments (50–150 p.p.m.) are quite high relative to MORBs and OIBs (~ 1

and 2–3 p.p.m., respectively). Boron concentrations of arc lavas are generally higher than those of magmas with comparable silica content from other tectonic settings¹³. Boron is strongly enriched in the altered portion of the subducting oceanic crust, with concentrations in the range 10–300 p.p.m., depending on the degree of alteration^{13–16}. In contrast to the large ranges in ^{10}Be and B concentrations, stable ^9Be shows little variation, with concentrations of $\sim 0.3\text{--}1.5 \text{ p.p.m.}$ in mid-ocean-ridge, ocean-island and arc basalts, as well as in ocean sediments and continental crust⁸.

The combination of ^{10}Be and B, with ^9Be as a normalizing element, is stronger than either data set alone. The suggestion that the subducted component may be homogeneous¹⁰ is greatly strengthened by the good two-component mixing lines shown by each arc. The extremely high $^{10}\text{Be}/\text{Be}$ and B/Be ratios implied for the subducted component allow us to reliably distinguish it from any type of mantle or continental crust, and to discuss its characteristics. The data also suggest that boron is not subducted to depth whereas ^{10}Be apparently is, and that boron is not stored in the sub-arc mantle. These observations raise the issue of chemical fractionation within the subducting plate as it traverses the region of magma generation, and suggest a methodology for evaluating recycling paths and times for elements subducted at convergent margins.

Results

Ratios of the cosmogenic isotope ^{10}Be to stable Be and B to Be are presented in Table 1. ^{10}Be was measured by accelerator mass spectrometry at the Tandem Accelerator Lab at the University of Pennsylvania following chemical separation of Be as previously described⁷. Concentrations of stable Be, essentially all ^9Be , were measured using an inductively coupled plasma technique reported in ref. 10. Boron concentrations were determined by prompt γ -ray-neutron activation at McMaster University following the procedure reported in ref. 17. Details of the analytical procedures are summarized in footnotes to Table 1. Analytical uncertainties are large for the low-concentration measurements (typically $\pm 15\%$, 1σ) but are small relative to the total range measured for arc lavas (more than an order of magnitude).

The results are plotted as $^{10}\text{Be}/\text{Be}$ and B/Be ratios in Fig. 1, essentially a ^{10}Be –B mixing diagram. As beryllium and boron seem to partition similarly in crystal–melt equilibria¹⁸, this normalization to beryllium minimizes or eliminates variations due to partial melting or crystal fractionation. Linear trends, which indicate mixing between materials of two distinct compositions (that is, end-members)¹⁹ are observed for lavas from each of four arcs. The good fit to a straight line (correlation coefficient $r^2 \geq 0.86$) observed for each arc indicates that the endmembers are approximately homogeneous in composition. The low- ^{10}Be , low-B end of the mixing trends is common to all arcs and has a composition typical of the mantle. The linear trends for the different arcs diverge from the origin, towards a range of values appropriate for subducted materials.

Samples from the volcanic front of each arc define a mixing line of slope characteristic to that arc. The relative position of

TABLE 1 $^{10}\text{Be}/\text{Be}$ and B/Be in island-arc lavas

	Year of eruption	Rock type	B* (p.p.m.)	$^{10}\text{Be}/\text{Be}^\dagger$ (10^{-11})	B/Be
Aleutians ($t=3.8$ Myr $\ddagger$)					
Augustine, 84-Au10	1976	And	9.6	0.7	14.5
Unimak, SAR-7	?	Bas	13	6.1	21.3
Akutan, Ak	1978	And	14	5.7	17.7
Bogoslof, Bog	1796	Sil And	15	9.0	5.9
	1927	Bas	3.7	8.6§	4.1
Umnak, UM 21	1946(?)	Bas	17	7.5	31.5
Kastochi, K81-71	?	And	11	14.6	26.2
Kanaga, KAN 5-8	1900	Bas	32	10.6	39.0
Cascades ($t=9.8$ Myr)					
Mt St Helens, DS6	?	Bas	4	0.5	3.6
DS72B	?	Bas	5.7	0.1	4.7
Indian Heaven, DS23B-1	?	Bas	2.9	—	—
DS61-80	?	Bas	2.2	0.3	4.4
Middle America ($t=2.3$ Myr)					
El Chichon, Zr-2	1983	—	17	0.6	7.7
Pacaya, Gu-P701	1961	Bas	8.2	10.5	16.4
Izalco, Sal-Iz108	1966	Bas	—	19.5	—
Boqueron, Sal-B21	1917	And	37	17.0	29.1
San Miguel, Sal-Sm7	1855	Bas	20	22.9	40.8
Cosignuina, Ni-Cos9a	1835	And	46	47.2	100
Telica, Ni-Tel	H	Bas	36	41.1	92.3
Momotombo, Ni-Mt101	1905	Bas And	25	41.5	64.1
Nejapa Nic-Ne-202	?	Bas	2.5	12.7	7.6
203	?	Bas	2.8	7.5	7.4
Masaya, Ni-Ms4	1772	Bas	25	59.9	41.7
Irazu, CR-I63g	1964	Bas And	15	1.4	10.3
Arenal, CR-Ar82	1982	Bas And	12	1.4	18.3
South America ($t=3.8$ Myr)					
San Jose, 281284-4	19th C	Sil And	20	1.0	9.6
Chillan, 060376-1	1930's	Dac	94	1.4	48.7
Antuco, 051177-7	1853	Bas	12	2.3	18.8
Villarrica, 280976-1	pre.	Bas	23	4.0	29.1
261185-2	1948	Bas	26	6.4	38.2
101275-1	1971	Bas	25	5.3	42.4
241185-3	1985	Bas	28	4.0	32.6
Mocho, 220281-1	1863	And	23	2.0	23.7
Osorno, 090185-1H	1835	Bas	15	3.7	26.8
Hokkaido ($t=2.8$ Myr)					
Rausu-dake, KAT-2	H	And	—	5.2	—
E-San	H	—	—	80.8	—
Komaga-take	H	And	—	11.4	—
Oshima-Oshima	1741?	Dac	—	3.0	—
Bismarck ($t=2.0$ Myr)					
Ulawun, NGV 305	1973	Bas	39	—	186
USNM 112516-11	1970	Bas	35	21.2	121
NGV 365	1973b	Bas	31	27.6	129
Bamus, NGV 361	1878-94	And	32	22.2	103
Pago, NGV 362	1911-23	Dac	21	14.7	39.6
Makalia, NGV 126	19th C	And	11	11.5	22.9
Garove, NGV 141	Hol	Bas	5.5	4.7	8.6
Langila, NGV 302	1973	Bas And	18	—	—
NGV 300	1975	Bas And	19	17.3	42.2
Long I, NGV 310	1973	Bas	11	12.0	18.3
Karkar, 74400050	1974	Bas And	—	13.9	—
Manam, PNG 34	1958	Bas	21	5.3	124
NGV 454	1964	Bas	23	—	—

* Details of technique given in ref. 17. Uncertainties are $\pm 10\%$ at $\text{B} > 10$ p.p.m. Uncertainty in B/Be ratio is typically $\pm 15\%$.

† Details of technique and ^{10}Be and ^9Be concentrations reported in ref. 10; ^{10}Be concentrations are normalized to in-house standard 482-CA = $2,111 \text{ atom g}^{-1}$, consistent with ref. 7. Reproducibilities (1σ) for measurements are better than: $\pm 15\%$, ^{10}Be ; $\pm 10\%$ for $\text{Be} \geq 0.3$ p.p.m.; $\pm 20\%$ for $\text{Be} \leq 0.3$ p.p.m.; $\pm 22\%$ for $^{10}\text{Be}/^9\text{Be}$ ratio.

‡ Calculated from geometry and convergence rates in refs 21, 32-36.

§ From ref. 8.

|| W.P.L., unpublished data.

samples along the mixing line is independent of silica content. A few points in Fig. 1 lie significantly far from the regression lines. Most of these are from centres located behind the volcanic front (Bogoslof, Makalia and Garove), where subduction times are longer. Vertical arrows indicate calculated $^{10}\text{Be}/^9\text{Be}$ ratios corrected for the additional ^{10}Be decay that occurs during these longer subduction times. The corrected points lie to the left of

the mixing line defined by samples from the volcanic front, indicating depletion of B relative to ^{10}Be with increasing depth to the subducting slab. Augustine volcano, eastern Aleutians, lies on the volcanic front in a region where the arc-trench gap is wide and subduction times are long (but the depth of the slab is similar to that elsewhere on the front); the corrected point falls nearly on the regression line, suggesting no boron loss

(Fig. 1a). The high-titanium lavas from Nicaragua differ from the adjacent low-titanium lavas²⁰ by having low B/Be and low $^{10}\text{Be}/\text{Be}$ ratios (Fig. 1b). The point for the Nevados de Chillan volcano in southern Chile (Fig. 1c) lies far from the trend of the others, perhaps reflecting shallow boron enrichment. Data for Manam volcano, from a region of arc-continent collision²¹ in the westernmost Bismarck arc, lie far from the straight line defined by data from volcanoes in the zone of active subduction, perhaps reflecting cessation of subduction and termination of the supply of young ^{10}Be -rich sediments to the zone of magma generation (Fig. 1d).

The well correlated ^{10}Be -B data are interpreted below in terms of a subduction contribution to arc magmas. The lavas are too young to have acquired their ^{10}Be signature through cosmic-ray bombardment or near-surface alteration^{7,9,10}. Crustal materials older than about 10 Myr are too old to impart a ^{10}Be enrichment and are generally too depleted in B to produce the observed arc trends. Near-surface hydrothermal fluids may mobilize boron (refs 12, 16), but are unlikely to mobilize sufficient ^{10}Be (ref. 22). Assimilation models require that lavas incorporate ^{10}Be -rich ocean sediments at shallow levels before significant crystallization^{9,10}. This is unlikely in general and is particularly implausible for the continental arcs. Moreover, assimilation is more likely to spoil systematics than to produce them. The results presented here indicate that B and ^{10}Be enter the arc source region together and behave coherently thereafter; the discussion below develops subduction models to explain these data.

Evidence for a subducted end-member

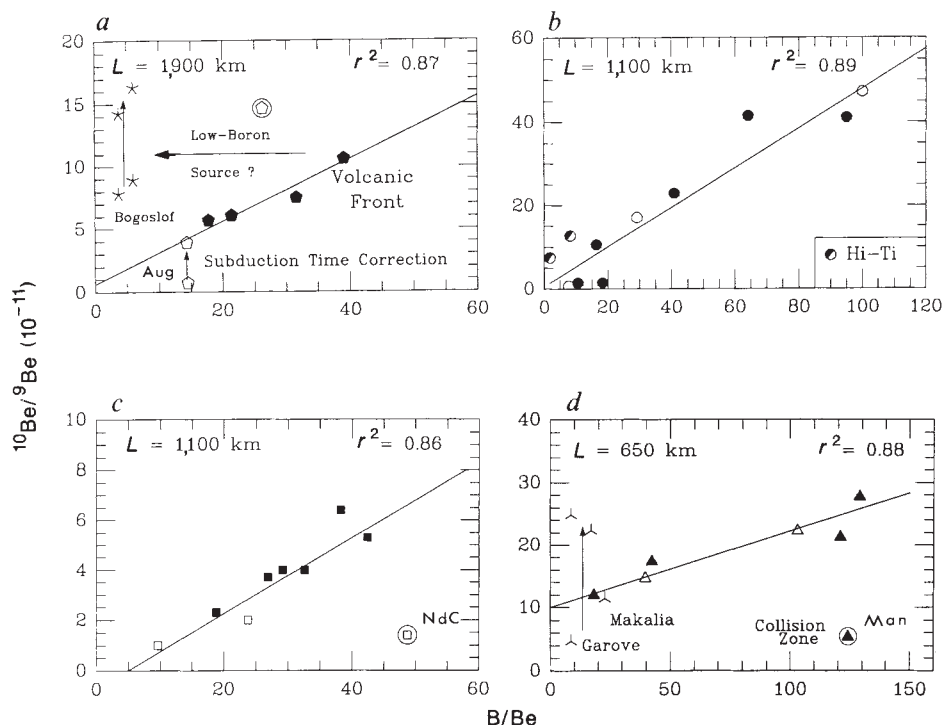
The arc trends from Fig. 1 are combined in Fig. 2, with fields for mantle and crustal reservoirs (including OIB, MORB, upper and lower continental crust) for comparison. All of these reservoirs are characterized by low $^{10}\text{Be}/\text{Be}$ ratios and by generally low B/Be ratios. Also shown are fields for high- ^{10}Be pelagic sediments and high-B altered oceanic crust. Because little data yet exist to define end-member characteristics, fields in Fig. 2 are drawn to cover about twice the range of reported data. The arc arrays always extend far beyond the range of mantle values; on the scale of variation seen in arcs, all variants of the mantle can essentially be represented by a point at the origin. The observed ranges for arcs also extend beyond those seen in lower

continental crust and in most upper crustal materials. The absence of the necessary high- ^{10}Be and high-B reservoirs in the mantle and crust strongly support the suggestion that a subducted component is involved in the formation of many arc magmas. The observation that data from virtually all types of mantle and crust lie near the origin on this diagram is important in explaining the apparent two-component mixing behaviour seen in arcs: a homogeneous subducted component establishes the ^{10}Be and B budget, and all other reservoirs (depleted mantle, enriched mantle, lower and upper continental crust) serve as undistinguished diluents.

The inferred composition of the subducted component for any arc must lie along an extension of the mixing line defined by the lavas. In theory, the subducted material may be pelagic sediments, altered oceanic crust, a mixture of the two, or melts or fluids derived from them. Several of these possibilities may be ruled out. The arcs shown in Figs 1 and 2 all have elevated ^{10}Be concentrations and therefore require the incorporation of young marine sediments; altered oceanic crust or old ^{10}Be -poor sediment alone are not acceptable end-members for the arc mixing arrays. B/Be ratios in bulk pelagic sediments (typically ~50-60) are too low to permit them to be end-members for the arc trends unless the lavas represent 50 to 90% sediment incorporation, and sometimes not even then (for example, Bismarck arc lavas have B/Be = 125). Melts of sediments will not have appreciably higher B/Be ratios and can also be ruled out. This result is in contrast to one of the models based on beryllium isotopes alone¹⁰, in which bulk sediments can satisfy the data. Fluid derived from pelagic sediments alone, with preferential partitioning of boron into the fluid (Case 1), is an acceptable end-member for the arc trends, as is a bulk mixture (or melts/fluids) of ^{10}Be -rich pelagic sediments and boron-rich altered oceanic crust (Case 2). Both mechanisms are illustrated schematically in Fig. 2.

It is difficult to specify the exact composition of the subducted materials, as the $^{10}\text{Be}/\text{Be}$ ratio is a function of age and stratigraphy of the subducted sediment column. Here we use $^{10}\text{Be}/\text{Be}$ atom ratios of $1,700 \times 10^{-11}$ and 200×10^{-11} . The high value corresponds to that measured in surface sediments of the central Pacific⁴ following correction for ^{10}Be decay during the 2.5-Myr subduction time (appropriate for Central America and the

FIG. 1 $^{10}\text{Be}/\text{Be}$ atom ratio ($\times 10^{-11}$) plotted against B/Be for a, the Aleutians; b, Central America; c, the Andes, Southern Volcanic Zone, Southern Chile; d, Bismarck arc. Closed symbols are basalts and basaltic andesites, open symbols are siliceous andesites and dacites, and skeletal symbols indicate samples from centres located behind the volcanic front. Different arcs are shown on different scales to permit comparisons between samples within an arc. L is the length of volcanic arc sampled. r^2 is linear-regression correlation coefficient; circled symbols and behind-the-front samples not included in linear regression. Vertical arrows connect measured values with calculated ratios corrected for ^{10}Be decay during extra subduction time for lavas from behind the volcanic front or from regions of unusually wide arc-trench gap (as at Augustine). Note extremely low B/Be ratios for behind-the-front lavas. Subduction times (given in Table 1) calculated using arc geometry and convergence rates taken from the literature^{21,32-36}.



Bismarck arc). The lower value is an estimated average for the entire sediment column of a subducting plate that is ~55 Myr old (similar in age to that subducting beneath the Aleutians, Central America and the Bismarck arc, and older than that beneath Southern Chile), also corrected for ^{10}Be decay during subduction. The value of 200 also corresponds to an ~7.5-Myr-old stratigraphic horizon. Clearly, older sediments and oceanic crust will have $^{10}\text{Be}/\text{Be}$ ratios as low as zero, but incorporation of such material will not produce the observed high ^{10}Be concentrations of many arc lavas.

Modelling the arc lavas

The possible effects of addition to the mantle wedge of a boron-enriched sediment-derived 'fluid' (Case 1) are shown in Fig. 3a (compare Fig. 4b in ref. 10). Tick marks indicate the amount of sediment-derived fluid added to the mantle. The precise mixing proportions are dependent on details of the models and are included for comparative purposes only; our most important conclusions are relatively independent of specific models. We calculate the B/Be ratio of the fluid assuming that 5% of the sediment-hosted beryllium and 70% of the boron is partitioned into the fluid. The beryllium partitioning is based on serpentine dehydration studies²³; boron is assumed to be slightly more compatible in a fluid than is rubidium in these experiments²⁴. This preferential partitioning of boron into a fluid is consistent with results from studies of hydrothermal systems²⁵. We assume that the $^{10}\text{Be}/\text{Be}$ ratio of the fluid is the same as that of the sediment, which implies that adsorbed ^{10}Be behaves like adsorbed boron and becomes incorporated in crystal sites during diagenetic and metamorphic recrystallization²⁶.

Mixing trends between the mantle wedge and fluids derived from surface sediments ($^{10}\text{Be}/\text{Be} = 1,700 \times 10^{-11}$), and between the mantle and fluids from deeper, older sediments ($^{10}\text{Be}/\text{Be} = 200 \times 10^{-11}$) bound the observed range of arc trends (Fig. 3a). When corrected for variation in subduction time, differences in the slope reflect differences in the $^{10}\text{Be}/\text{Be}$ ratio (and hence average age) of the sediments involved in magma generation. In Fig. 3a, subduction times for Central America and the Bismarck arc are similar (~2.5 Myr) and the slopes may be compared directly. Subduction times are longer in the Aleutian and southern Chile arcs; correction to 2.5 Myr would cause the slope of the Aleutian trend to become comparable to that of Central

America, whereas the slope of the southern Chile trend would become intermediate between Central America and the Bismarck arc. In arcs of radically different thermal structure (such as the Cascade and Mariana arcs), some variation in boron and beryllium fluxes might also be expected as a result of differing mineral stabilities in the downgoing slab^{27,28}. As shown by calculated mixing proportions in Fig. 3a, the longer the arc trend, the greater the maximum amount of sediment-derived fluid involved in magma generation. In this model, lavas from the Aleutians and southern Chile have the least amount, relatively, of a sediment component. Short trends for these arcs could also reflect higher boron and beryllium concentrations in the mantle source and thus less of a compositional shift induced by the subducted component^{29,30}. Alternatively, addition of relatively large amounts of low- ^{10}Be and low-B continental crust could dilute the subduction-zone signature, causing a point to shift down the mixing line back towards the origin.

The high correlation coefficients in Fig. 1 indicate that the subducted component, fluids in this model, is essentially homogeneous. This was also inferred, albeit less conclusively, from the beryllium isotope data alone¹⁰. This is a striking result given that the $^{10}\text{Be}/\text{Be}$ ratio decreases down a sediment column as a function of age and stratigraphic level. If high correlation coefficients persist when larger data sets are available, this observation requires that the B/Be and the $^{10}\text{Be}/\text{Be}$ ratio (and hence mean age) of the sediment involved in magmatism be approximately constant along the arc. This could be achieved if: (1) fluids are everywhere derived from the same stratigraphic level, or (2) the sediment column is well homogenized mechanically during subduction, or (3) the process of fluid formation and migration is an effective agent for homogenizing the sediment column. In the latter two cases, the constant $^{10}\text{Be}/\text{Be}$ and $^{10}\text{Be}/\text{B}$ ratios implied for the sedimentary component suggest that the mean age and stratigraphic succession of sediments actually subducted to depth are similar along the length of an arc.

Addition to the mantle of a subducted component, which is itself a mixture of high- ^{10}Be sediments and high-B altered MORB (Case 2), is illustrated in Fig. 3b. This model was not previously developed¹⁰, as its plausibility is indicated by the combined B-Be data set. Curves at the high B/Be end of the diagram show bulk mixing between altered MORB and sediments, for both 'surface' ($^{10}\text{Be}/\text{Be} = 1,700 \times 10^{-11}$) and 'deeper' sediments (200×10^{-11}). The implied end-member for each arc is given by the intersection of the extrapolated arc trend (shown as a dashed line in the case of the Bismarck arc) and the appropriate mixing curve between sediment and altered MORB. The composition of the implied arc end-member (shown as small circles on the extension of the Bismarck trend) varies little, even though the $^{10}\text{Be}/^{9}\text{Be}$ ratio of the sediment varies from 1,700 to 200. The calculated proportion of altered slab to sediment does differ significantly between the two curves, but is always dominated by altered MORB. In this model, the slope of an arc trend is related to the $^{10}\text{Be}/\text{Be}$ ratio of the sediment and the proportion of sediment to altered slab in the subducted component. If the ^{10}Be ratio of the sediment subducted to depth is approximately constant, the good correlation coefficients of Fig. 1 indicate that the proportion of sediment to altered slab in the subducted component is approximately constant and apparently well regulated. A less plausible alternative explanation of the good correlation coefficient for a given arc is that there is a trade-off between the amount of sediment in the subducted component and the $^{10}\text{Be}/\text{Be}$ ratio of the sediment, so that a constant composition in the end-member is retained. Again, distance from the origin on the mixing trend indicates amount of the subducted component. As shown by the slab-sediment mixing lines, the altered-MORB contribution to the subducted component in the different arcs varies from 70 to 99%, depending on the age of the sediment involved.

To satisfy the arc data, this model requires preferential involvement of the altered fraction of the subducting oceanic

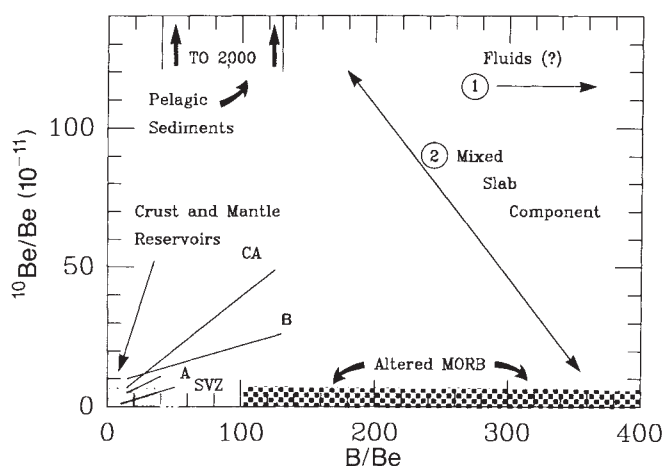


FIG. 2 $^{10}\text{Be}/\text{Be}$ atom ratio plotted against B/Be showing arc trends from Fig. 1, fields for mantle and crustal reservoirs (MORB, OIB, upper and lower continental crust^{8,13,37-39}, altered MORB^{11,14,16} and pelagic sediments^{4-6,8,13,40}). $^{10}\text{Be}/\text{Be}$ ratios for pelagic sediments have been corrected for ^{10}Be decay during 2.5-Myr subduction time. Also shown schematically are two mechanisms for producing the high- ^{10}Be and high-B end-members implied by the arc mixing trends through (1) preferential partitioning of B into a sediment-derived fluid and (2) mixing of high- ^{10}Be sediments and high-B altered slab.

crust, either through partial melting (at the slab-mantle interface, for example) or through derivation of a fluid from this region. Complete melting of the bulk slab would not produce a component with a high enough B/Be ratio to be an end-member for many of the arc trends. Mixing of fluids derived from altered MORB and from sediments would produce curves similar to those of Fig. 3b, but at much higher B/Be ratios. Such a fluid, characterized by lower element abundances than solid or melted mixtures, would need to be incorporated in larger proportions than those calculated in Fig. 3b for addition of a bulk sediment/slab mixture. Combination of the Be-B data with isotope and trace-element studies of subduction-related lavas will be necessary to distinguish between the models sketched in Fig. 3a, b.

Implications of Be-B systematics

Whichever model proves to be correct, the requirement that the subducted end-member in an arc be homogeneous with respect to the $^{10}\text{Be}/\text{Be}$ and B/Be ratios suggests that it may also be homogeneous with respect to many other isotope and trace-element characteristics. If so, simple mixing between the subducted component and a homogeneous mantle, without the involvement of crust, would produce linear mixing trends for other isotope or incompatible-trace-element ratios considered. Departure from such simple mixing behaviour may then reflect either pre-existing variability in the mantle, complexities in mantle modification and melting, or the effect of crustal involvement, but not heterogeneity in the subducted component.

One of the striking features of Fig. 2 is that all arc trends converge on the origin. This is paradoxical because continued sediment addition to, or metasomatism of, the arc source region will increase the $^{10}\text{Be}/\text{Be}$ and B/Be ratios of the mantle. The high- ^{10}Be signature will decay with time, leaving a high and variable B/Be ratio in the mantle as a relict of earlier subduction modification. Addition of a homogeneous subduction component to a heterogeneous mantle would produce mixing fields, not lines. If the modified mantle were well stirred, mixing lines would result, but with elevated non-zero B/Be intercepts. The absence of such boron enrichment in Fig. 2 implies that the residence time of boron in the sub-arc mantle is comparable to the ^{10}Be decay time, ~ 5 Myr. This may indicate that boron is the ultimate fugitive element, recycled quickly through convergent margins, and spending most of its life in the lithosphere and especially in the hydrosphere. Alternatively, convection may remove subduction-modified mantle at about the same rate that it is being formed above subduction zones. If this is so, this high B/Be mantle may be subsequently tapped in back-arc volcanism, post-subduction magmatism, and volcanism from high- μ (elevated $^{238}\text{U}/^{204}\text{Pb}$) sources at ocean islands, an issue under investigation³¹. The low B/Be ratios observed in behind-the-front volcanoes suggests, however, that boron is not recycled to depth. Elements such as caesium, rubidium and uranium may behave similarly.

The models discussed here may be compared with those based on beryllium isotope ratios and beryllium concentration data alone¹⁰. The approaches differ in that: (1) reservoirs (enriched mantle, depleted mantle, MORB, OIB and most continental materials) with indistinguishable B/Be ratios can be distinguished on the basis of beryllium concentrations; and (2) variations in element concentrations reflect processes as well as sources. Whereas both studies emphasize the compositional effects of subduction modification of the mantle, the use of concentration data in the earlier study shows that there are further complexities. The earlier results indicated that the type of subduction-related modification of the mantle discussed here is followed by processes that are not apparent on the plot of $^{10}\text{Be}/\text{Be}$ against B/Be. These second-stage processes¹⁰ may involve mixing of melts in the mantle, mixing of crustal materials with mantle-derived magmas, or a melting process in which the extent of melting increases as the amount of a subduction

component (fluid?) in the mantle increases. By contrast, the approach followed here allows us to isolate the subducted component, discuss some of its characteristics, and link those to the geology and geophysics of convergent margins. Larger sample sets and broader data sets are necessary to distinguish among the possibilities and arrive at a quantitative understanding of magmatism at convergent margins. The good systematics that emerge from these two studies indicate, however, that subduction recycling and magma formation are linked and apparently well regulated.

Discussion

^{10}Be and B budgets in arc lavas are almost completely determined by a recycled subduction component. As a consequence,

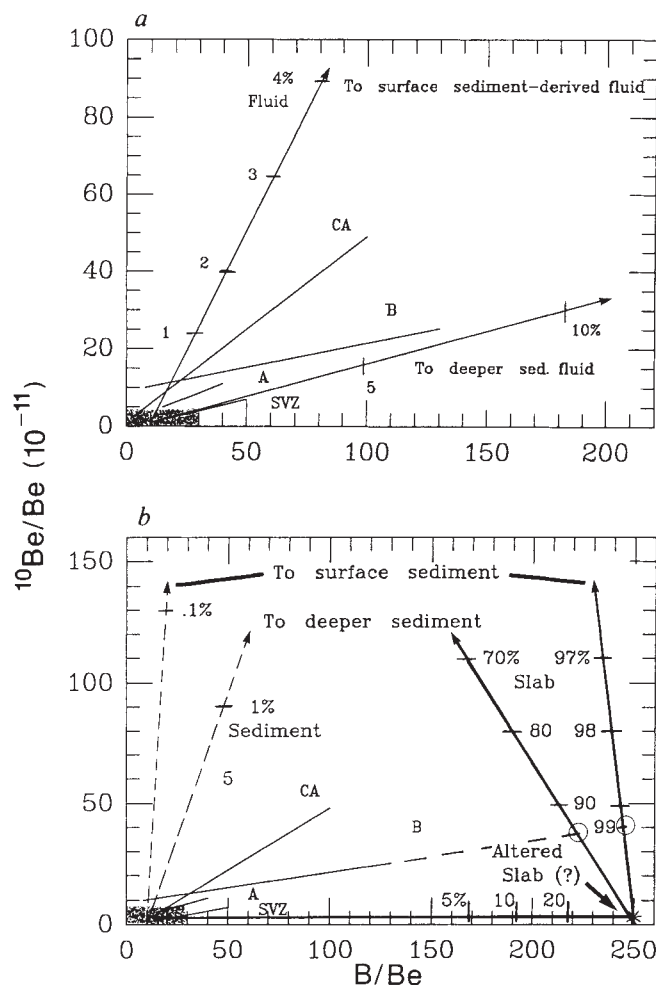


FIG. 3 a, Mixing calculations showing effect of adding hypothetical sediment-derived fluid to the mantle. Sediment-derived fluid is taken to have 0.07 p.p.m. Be and 82 p.p.m. B, following ref. 23 and assuming 1.3 p.p.m. Be and 125 p.p.m. B in the original sediment^{8,39,40}. $^{10}\text{Be}/\text{Be}$ ratio for 'surface' sediments ($\approx 1,700$) corresponds to surface Pacific sediments, 'deeper' sediments are ~ 5 Myr old, with the $^{10}\text{Be}/\text{Be}$ ratio (≈ 200) corrected for ^{10}Be decay during a 2.5-Myr subduction time in both cases. Tick marks indicate percentage of sediment-derived fluid in mantle source region with 0.05 p.p.m. Be and 0.15 p.p.m. B. b, Calculations show bulk mixing between altered slab and 'surface' and 'deeper' sediment to form a mixed subducted component, shown in heavy solid lines at right of diagram. Tick marks indicate percentage of slab in slab-sediment mixture. Also plotted are mixing curves showing the effects of sediment addition to the mantle (nearly vertical dashed lines at the left of the diagram) and of addition of an altered slab component (horizontal solid line). Tick marks indicate percentage of sediment and altered slab, respectively, in the mantle; proportions are calculated assuming that sediment and altered slab are added as 30% partial melts with Be and B behaving as completely incompatible elements. B concentration of altered slab taken to be 125 p.p.m., Be = 0.5 p.p.m. (refs 8, 13) and mantle and sediment concentrations are as in a.

these two tracers are largely insensitive to variations in the chemistry of the sub-arc mantle and to crustal contribution. The corollary is that beryllium and boron may be used to characterize the material recycled during subduction and magmatism. The most striking conclusion is that the subducted component in each arc, probably 'fluid' derived from sediments (with or without the altered oceanic crust) is homogeneous within an arc. This observation constrains the recycling process to be surprisingly well regulated. That large age-dependent variations in $^{10}\text{Be}/\text{Be}$ ratios in the sediment column can apparently be homogenized suggests that the subducted component may be

homogeneous with respect to other chemical tracers. If so, then the frequent complexities observed in other aspects of arc geochemistry could reflect pre-existing heterogeneity of the mantle, complications in subduction modification and melting of the mantle, or interaction of magmas with the crust. The Be-B systematics show that the sub-arc mantle has no memory of boron enrichment imposed during earlier subduction, suggesting that boron is flushed quickly through the mantle and raising the possibility that different elements may have characteristic and distinct recycling patterns during subduction. □

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Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins

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Analysis of complementary DNA for human erythrocyte ankyrin indicates that the mature protein contains 1,880 amino acids comprising an N-terminal domain binding integral membrane proteins and tubulin, a central domain binding spectrin and vimentin, and an acidic C-terminal 'regulatory' domain containing an alternatively spliced sequence missing from ankyrin variant 2.2. The N-terminal domain is almost entirely composed of 22 tandem 33-amino-acid repeats. Similar repeats are found in yeast and invertebrate proteins involved in cell-cycle control and tissue differentiation.

ANKYRINS constitute a family of proteins that control interactions between integral membrane components and cytoskeletal

elements. Erythrocyte ankyrin (protein 2.1) is the best-characterized isoform. It attaches the spectrin skeleton (membrane skeleton) to band 3, the anion-exchange protein¹⁻⁷. This interaction protects red cells from circulatory shear stresses and helps maintain their unique biconcave shape.

A different ankyrin has been purified from brain tissue⁸ and there are hints that other ankyrins exist⁹⁻¹¹. Ankyrins are found in many other, and perhaps all, cells⁸⁻¹⁷. They have been provisionally subdivided into 'erythrocyte' and 'brain' isoforms on the basis of their immunological similarity to the two well-characterized ankyrins¹⁷. Erythrocyte-type ankyrins are typically confined to specific membrane domains^{14,17} and bind integral membrane proteins, such as the (Na⁺ + K⁺)-ATPase¹⁸⁻²⁰ and the voltage-dependent Na⁺ channel^{21,22}, that must be polarized to function properly. Brain-type ankyrins are more widely distributed and bind to different integral proteins¹⁷, none of which have been identified.

Erythrocyte ankyrin contains two principal chymotrypsin-resistant domains that were originally judged to have relative

molecular masses of 90,000 (M_r 90K) and 72K on the basis of their mobility on SDS gels⁶. The studies described here, however, demonstrate that these domains actually have M_r s of 89K and 62K. Analogous 83K and 55K domains are produced by digestion with trypsin^{23,24}. Brain ankyrin has a similar structure⁸. The 89K domain binds the erythroid anion exchanger^{25,26} and tubulin^{9,25}. The 62K domain binds spectrins^{8,27} and vimentin²⁸. The loci of other binding sites, including those for the (Na^+ + K^+)-ATPase¹⁸⁻²⁰, the Na^+ channel^{21,22} and protein 4.2 (ref. 29), are unknown. Additional 'regulatory' sequences modulate the known binding functions^{17,30}. One is released by the Ca^{2+} -stimulated protease, calpain. The other is missing from a variant of erythroid ankyrin called ankyrin 2.2 (or protein 2.2). Ankyrin 2.2 is particularly interesting because it is an 'activated' molecule with the ability to bind to all brain and erythroid ankyrin sites, and to additional sites of its own¹⁷.

Ankyrins therefore facilitate and probably control interactions between integral membrane proteins and cytoskeletal elements. To begin to understand these functions, we first determined the structure of erythrocyte ankyrin.

Complementary DNA cloning

We cloned ankyrin from a human reticulocyte cDNA library³¹ to ensure isolation of the erythrocyte isoform. We obtained a single clone, pAnk1B, from immunological screening of 1.2×10^6 recombinants. We detected twenty-six clones on multiple

rescreenings' and subcloned and sequenced six overlapping clones (Fig. 1a). These overlapping clones span a distance of 7,252 base pairs (bp) and include pAnk58 (1-1,593), pAnk42B (965-2,422), pAnk37 (2,162-4,281), pAnk15 (3,155-5,616), pAnk1B (4,244-4,621, and 5,108-6,134) and pAnk22 (5,771-7,252).

The parent clone, pAnk1B, detects a prominent broad band of ~7 kilobases (kb) on northern blots of human reticulocyte or bone marrow poly(A)-selected RNA (Fig. 1d). This resolves into two closely spaced fuzzy bands of ~6.8 and 7.2 kb on lightly exposed autoradiographs (data not shown). A third species of 8.9 kb is present in bone marrow RNA but absent in reticulocytes (Fig. 1d), indicating a developmentally modulated switch in gene expression or splicing.

Complementing DNA sequence

Translation almost certainly begins at the first ATG of the nucleotide sequence (Fig. 2). There is an in-frame termination codon 12 bp upstream, and the surrounding nucleotides conform reasonably well to the Kozak consensus sequence for translation start sites³². The sequence immediately after this methionine is identical to the N-terminal sequence of native ankyrin. Presumably the initiator methionine is removed during synthesis exposing the underlying proline. This is a well known reaction, although proline is not one of the amino acids typically exposed³³.

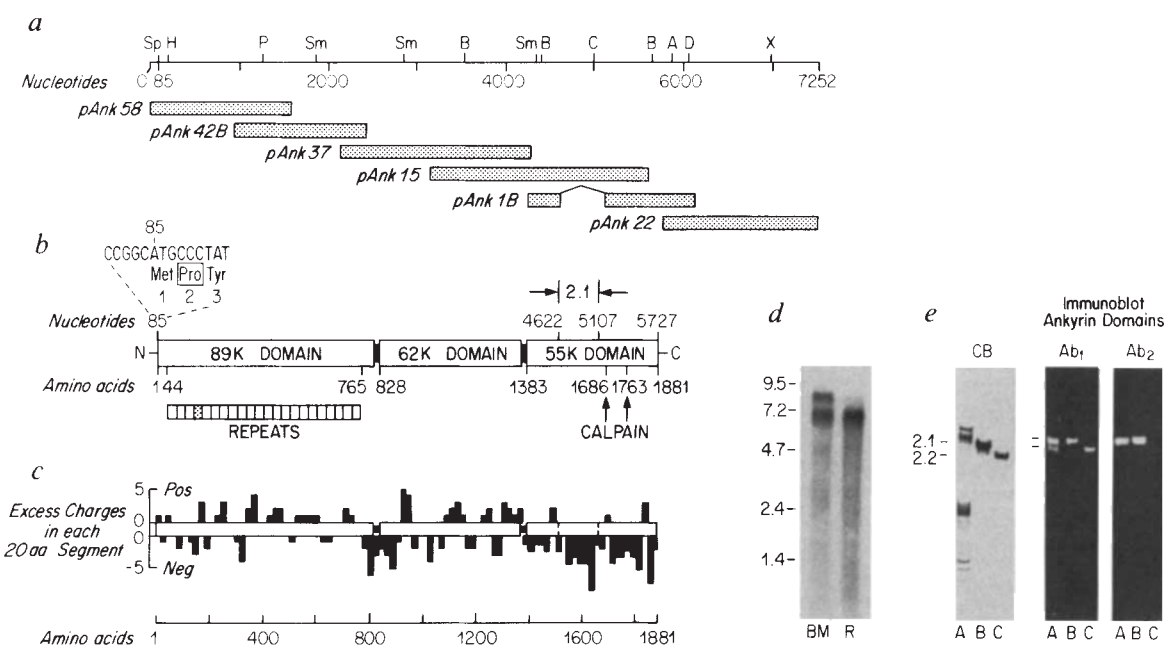


FIG. 1 Organization of human erythrocyte ankyrin cDNA and protein. *a*, Restriction-enzyme map of ankyrin cDNA. The combined sequences of the six overlapping cDNAs extend 7,252 bp. Restriction sites: *AccI* (A), *Bam*HI (B), *Clal* (C), *DraI* (D), *Hind*III (H), *PvuI* (P), *SmaI* (Sm), *SphI* (Sp) and *XhoI* (X). *b*, Structure of ankyrin. The probable translational start site is shown expanded (left); the N-terminal proline (residue 2) of the mature protein is boxed. The processed protein contains 1,880 amino acids (M_r 206,172). The 89K domain begins at the N terminus and probably extends to residue 827. It is mostly composed of related 33-amino-acid sequences (open boxes). The fourth such sequence (shaded) is shorter and less homologous. The 62K domain begins at residue 828 and extends to at least residue 1,367. It is followed by a protease-sensitive 'regulatory' domain (M_r 55K) which probably begins at either residue 1,383 or 1,387 (or both) and extends to the C terminus. This domain contains two calpain cleavage sites and an alternatively spliced sequence, designated 2.1, that is missing from the clone pAnk1B product and from ankyrin 2.2. *c*, Distribution of charges in ankyrin. The excess number of positive (Pos; Arg, Lys; upper bars) or negative (Neg; Glu, Asp; lower bars) amino acids in each 20-residue segment is shown. *d*, Northern blot of poly (A)-selected RNA from human bone marrow (BM) or peripheral blood reticulocytes (R). *e*, Immunochemical identification of the

2.1 sequence. Purified ankyrin containing both ankyrin forms 2.1 and 2.2 was separated by SDS-PAGE and stained directly with Coomassie blue (CB) or transferred to nitrocellulose, stained with Ab1 or Ab2 antibody, and counterstained with ^{125}I -labelled protein A⁶⁴. A negative image of the resulting autoradiographs is shown. Ab1 was raised against the larger calpain fragment (residues 1,686-1,881) and recognizes both 2.1 and 2.2 forms, whereas Ab2, which was directed against a synthetic peptide (residues 1,601-1,622) contained in the 2.1 sequence, only detects the 2.1 form of ankyrin. Abbreviation: aa, amino acids.

METHODS. Clone pAnk1B was retrieved from a human reticulocyte λ gt11 library³¹ by immunochemical screening⁶⁵ with a polyclonal rabbit antiserum. The library was rescreened repeatedly with clone pAnk1B or with clones derived from it. Plaque-purified DNA was subcloned into Bluescript-KS(+) (Stratagene) or pGEM-7Zf(+) (Promega). Both strands of the six overlapping clones shown in *a* were entirely sequenced by the dideoxy-chain-termination method, using T7 DNA polymerase (Sequenase, United States Biochemical Corp.)⁶⁶ and nested deletions produced by the *Exo*III-S1 nuclease technique⁶⁷. The cDNA sequence data were compiled and analysed using the University of Wisconsin Genetics Computer Group (UWGCG) programs⁶⁸.

[illegible]

◀ FIG. 2 Nucleotide sequence and deduced amino-acid sequence (below) of cloned human erythrocyte ankyrin cDNAs. Sequences that match amino-acid sequences obtained from purified ankyrin or its isolated domains are underlined. Gaps in these sequences are indeterminate residues. The N-terminal proline of the mature protein, the deduced boundaries of the 89K, 62K and 55K structural domains (see text), the observed boundaries of the 2.1 sequence that is missing in ankyrin 2.2, and the locations of the two calpain cleavage sites, are shown. The arrowheads, R1–R22, mark the beginning of each of the 22 consecutive repeated sequences. Polymorphisms due to differences between the translated cDNA sequence and the amino-acid sequences of isolated peptides are shown below the translated sequence at residues 21, 750, 845, 1,286 and 1,392. The boxed nucleotides in the 3' untranslated region enclose a TG-rich sequence (6,015–6,062), an A-rich sequence (6,134–6,156), 14 AC-repeats (6,304–6,331), and an AATAA sequence (6,729–6,734). The nucleotide sequence is registered with GenBank (accession number X16609).

The deduced sequence of ankyrin extends for 1,880 amino acids from the N-terminal proline (corresponding to an M_r of 206,144; pI 5.95), and the 5' and 3' untranslated regions cover at least an additional 84 bp and 1,525 bp, respectively. There is no poly(A) tail or polyadenylation signal at the 3' terminus of the cloned cDNA (although an AATAAA sequence occurs at position 6,729–6,734). But the messenger RNA cannot be much larger because the size of the largest reticulocyte RNA species (7,200 bp) corresponds closely to the total length of the cDNA clones (7,252 bp).

Verifying that the cDNAs encode ankyrin

We verified the identity of the ankyrin clones by comparison with N-terminal amino-acid sequences of 34 peptides isolated from proteolytic digests of erythrocyte ankyrin or its chymotryptic domains. Each of these sequences was found in the translated ankyrin cDNA sequence (Fig. 2, underlined amino acids). Five polymorphisms were detected by variations between the peptide sequences and the translated cDNA sequence (Fig. 2). One of these was also found for two of the cDNA clones (residue 1,286: Glu for pAnk37; (GAG codon), Asp for pAnk15 (GAC codon).

Ankyrin contains three structural domains

The deduced ankyrin sequence is divided into three regions corresponding to the domains defined by chymotrypsin cleavage. The 89K and 62K domains begin at residues 2 and 828, respectively, as judged by N-terminal sequencing of the isolated domains. The 89K domain extends to at least residue 753, because all of the peptides matching the deduced sequence to that point are derived from the isolated 89K domain. Similarly all peptides between residues 828 and 1,367 are derived from the 62K domain, and all peptides beyond residue 1,382 are isolated from native ankyrin under digestion conditions in which the 89K and 62K domains are not released. Therefore the 89K domain ends somewhere between residues 753 and 828, and the 62K domain ends somewhere between 1,367 and 1,382. There are no strong chymotryptic sites in either interval, indicating that each domain extends to the beginning of the next. This deduction is supported for the 89K domain by the fact that antibodies raised to its putative C-terminal peptide recognize the domain (V.B., unpublished observations). The N-terminal 89K domain therefore probably contains 827 residues (M_r 89,021; pI 8.0) and is released by cleavage of Phe 827–Lys 828, whereas the centrally positioned 62K domain probably contains 555–559 amino acids (M_r 61,991–62,505; pI 6.8–7.0) and terminates by cleavage of the Leu 1,382–Ala 1,383 bond, the Tyr 1,386–Ser 1,387 bond, or both.

The remaining 495–to–499 C-terminal amino acids (M_r 54,682–55,195; pI 4.1) form a functional domain that regulates the binding of ankyrin to spectrin and the anion-exchange protein³⁰.

The function of ankyrin is also regulated by phosphorylation^{34–36}. *In vitro*, up to seven Ser–Thr phosphates are added to ankyrin by the erythrocyte membrane cyclic AMP-independent casein kinase I³⁴. This abolishes the preferential

binding of unphosphorylated ankyrin to spectrin tetramers and oligomers rather than to spectrin dimers^{34,35}, and reduces the capacity of ankyrin to bind band 3, the anion-exchange protein³⁶; but it is difficult to identify the phosphorylation sites, because the specificity of casein kinase I is not well defined. Erythrocytes also contain casein kinase II³⁷ and cAMP-dependent protein kinases³⁸, among others, and there is some evidence that these enzymes act on ankyrin³⁹. Potential sites for casein-kinase-II phosphorylation (X-Ser/Thr-X-X-Glu/Asp-X-X, where some of the residues denoted by X are acidic and none are basic)⁴⁰ exist, particularly at amino-acid positions 809, 817, 1,042, 1,666 and 1,859. Sites favoured by cAMP-dependent kinases (Arg-Arg-X-Ser/Thr)⁴¹ are present at positions 834, 1,378 and 1,500.

Ankyrin is also acylated by palmitic acid^{42,43}, and the rapid turnover of fatty acid indicates a regulatory role⁴³. Unfortunately, the affected cysteines are unknown and are difficult to predict, because no consensus sequence for palmitoylation has been identified.

Ankyrin 2.2 lacks some of the regulatory domain

The central third of the C-terminal 55K domain contains a 486-bp sequence that is present in one of the clones (pAnk15) and missing from an overlapping clone (pAnk1B) (Fig. 1). The borders of the inserted sequence are similar to splice donor- and acceptor-sites⁴⁴, particularly the 3' end, indicating that the insert arises from alternative splicing. The sizes of the predicted RNAs (6.77 and 7.25 kb) are compatible with the sizes of the two ankyrin RNAs observed on lightly exposed northern blots (6.8 and 7.2 kb) (Fig. 1d). The extra in-frame sequence encodes a polypeptide of M_r 17,283 that is highly acidic (pI 3.7, Fig. 1c). An antibody to a peptide in the insert stains full-length ankyrin (protein 2.1) but not a smaller variant called ankyrin 2.2 (Fig. 1e). By contrast, an antibody to a C-terminal peptide stains both ankyrins (Fig. 1e). Because the two variants only differ slightly on peptide maps^{4,5,29}, they are probably identical except for the presence (protein 2.1) or absence (protein 2.2) of the insert. The difference in their apparent M_r s on SDS gels (29K)³⁰ is greater than the size of the insert (17.3K); but the insert is so acidic that it probably binds detergent poorly, which would decrease the mobility of protein 2.1 on SDS gels relative to that of protein 2.2.

The absence of this insert in ankyrin 2.2 'activates' the protein and enhances binding to spectrin and to sites on membranes, especially kidney membranes^{17,30}. By contrast, cleavage by calpain after His 1,685 and Trp 1,762, releases two acidic peptides (M_r 22,008 and 13,539; pI 4.1–4.2) and partially 'deactivates' ankyrin, muting its affinity for the erythroid anion exchanger³⁰. The exquisite protease sensitivity of the 55K region (V.B., unpublished observations) implies it is less compact than the 89K and 62K domains. In particular, hydrodynamic measurements indicate that the C-terminal peptide released by calpain could extend from the molecule like a tail, because the frictional ratio of the remnant after calpain treatment is considerably less than that of native ankyrin³⁰.

Ankyrin repeats in the 89K domain

The 89K domain is almost entirely composed of a repeated sequence motif. This is easily detected by comparing the ankyrin amino-acid sequence with itself using a dot-matrix analysis (Fig. 3a). About the first 40 and last 60 amino acids are unique, but the core of the domain, from residues 44 to 765, is composed of 22 successive repeats. Each repeat contains 33 amino acids, except the fourth, which contains only 29 amino acids. There are no significant repeated sequences in other areas of the molecule.

The repeats are aligned in Fig. 3b. Fifteen of the 33 positions are well conserved, that is, only one amino acid or two closely related amino acids are found at the same position in two-thirds or more of the repeats. These form a signature consensus

sequence -G-TPLH-AA--GH---(V/A)--LL--GA--(N/D)--- (single-letter amino-acid code). As a first approximation, the repeats can be viewed as a series of conserved and 'variable' amino acids. The latter are marked by hyphens in the consensus sequence. But many of the variable residues are partially conserved. For example, amino acids in positions 3, 8, 15, 17 and 20 are hydrophobic. Those in positions 11, 16, 19, 23 and 24 are polar, and two of these, 11 and 24, are predominantly basic. In other positions, one or two specific amino acids dominate, but not to the extent that they do in the main consensus positions. These include positions 1 (Asn or Asp), 27 (Ser or a polar residue), 28 (Pro or a hydrophobic residue), 30 (Ala), 32 (Thr or Ser) and 33 (Lys). Positions 12 and 31 are the least conserved.

One important issue is how the repeats should be phased, because they do not start or end abruptly (Fig. 3b). The phasing shown in Figs 2 and 3 is chosen based on limited genomic sequencing (W. T. Tse, S.E.L. and B. G. Forget, unpublished observations); but a more complete analysis of the erythrocyte ankyrin gene is required to confirm this assignment.

The ankyrin repeats were presumably formed by successive gene duplications. We looked for patterns that would reveal some of the evolutionary steps by using a matrix that compared the similarity of the repeats. This approach was successful with spectrin⁴⁵, which contains repeated segments of 106 amino acids; but it was not informative with ankyrin, even though the repeats in ankyrin are more homologous than those in spectrin: $37.0 \pm 7.6\%$ (s.d.) identical to each other on average (range, 12–58%).

Structural-prediction algorithms (Chou-Fasman and Garnier-Osguthorpe-Robson) give similar results, whether applied to individual repeats and then averaged, or applied to a consensus repeat derived using the UWGCG program PROFILE (the sequence labelled 'ANKREPEAT' in Fig. 4b). Both approaches predict main structural elements between ANKREPEAT residues 4–11 and 16–23, framed by beta turns (residues 12–15, 24–26, and 32–2) and connected by amino acids of unspecified structure (residues 27–32). The first element is calculated to be an α -helix in 18 of the 22 repeats and in the consensus sequence. The second element could be either an α -helix or a β -sheet structure, judging from analysis of individual

repeats; it is predicted, however, to be helical in the consensus sequence. Both of the putative helices would be highly amphipathic, with all of the polar amino acids clustered on one face (residues 4, 7 and 11 of helix 1; and 16, 19 and 23 of helix 2). If they align antiparallel, as suggested by the short turn between them, and if the polar faces are both on the same side (for example, towards the solvent), then four of the most conserved residues would lie in the contact region between the two helices (residues 6 and 10 of helix 1, and residues 18 and 22 of helix 2).

The similarity of the repeats indicates that they would be isotropically organized and have similar contacts with each other and with the surrounding solvent. To predict the organization of the repeats it is necessary to know the shape of the 89K domain. A globular shape is probable because hydrodynamic and microscopic data indicate that ankyrin is globular, except for the proteolytically sensitive regulatory domain³⁰. For a globular domain, isotropic subunits could be arranged as a sphere, regular polygon or compact helix (cylinder). A helix would allow the number of repeats to vary among ankyrins or ankyrin-related proteins (see below) without altering the contact points or conformation of individual repeats. This is not so for a sphere or polygon.

Individual repeats or groups of repeats could form the binding sites for integral membrane proteins and tubulin. Preliminary data support this hypothesis for the anion exchanger (L. Davis, J. Davis, S.E.L. and V.B., unpublished observations), but no information is available for other integral proteins. Presumably the variable residues in each repeat provide the specificity required.

We searched the National Biomedical Research Foundation protein database (versions 20 and 21) and the GenBank nucleotide database (version 60) using a consensus sequence of the erythroid ankyrin repeats and found very similar repeats in several invertebrate, yeast and viral proteins (Fig. 4). Multiple searches with other portions of the ankyrin sequence revealed no other significant homologies.

The 'ankyrin-like' repeats are easily detected on dot-matrix plots (Fig. 4a) and closely resemble the repeats in erythroid ankyrin (compare Figs 3b and 4b). The principal differences

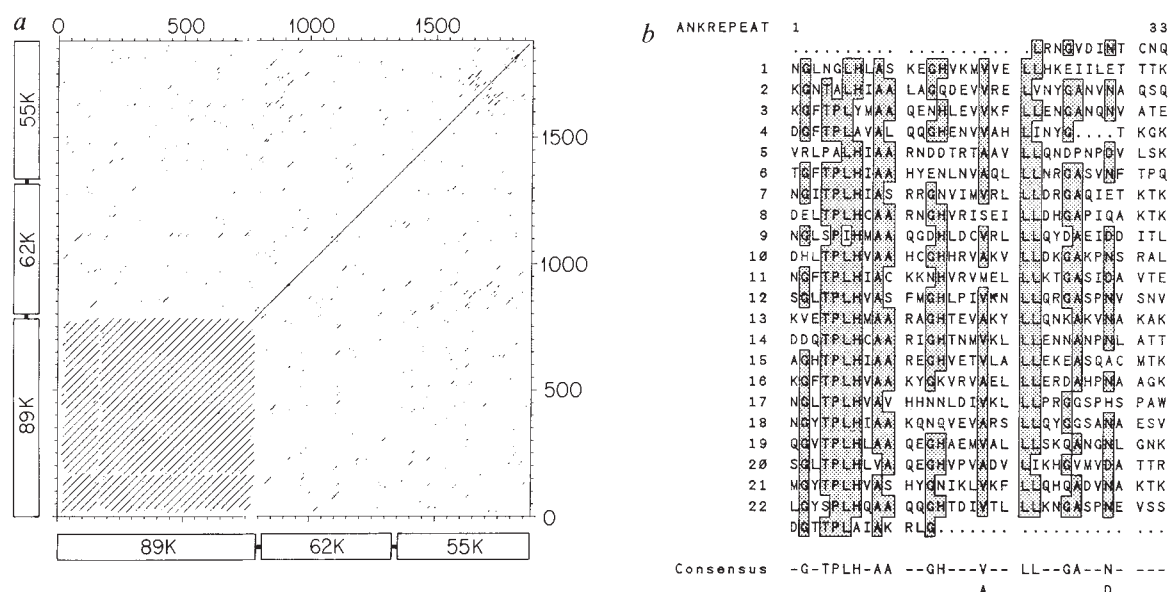


FIG. 3 Repeating sequences in ankyrin. *a*, Dot-matrix analysis. The complete amino-acid sequence and the location of the three main structural domains of ankyrin are represented on both the horizontal and vertical axes. The dot-matrix plot was prepared using the UWGCG programs COMPARE and DOTPLOT with a window of 30 and a stringency of 15. Segments of 30 amino acids from the horizontal axis were compared with segments from the vertical axis, and a dot was placed in the appropriate position whenever the total score⁶⁹ of the aligned sequences was ≥ 15 . The parallel lines on

each side of the diagonal line of self-identity represent 22 very similar repeating segments. Each is of 33 amino acids, except for repeat 4 (29 residues), which produces the discontinuity shown. They occupy almost the entire 89K domain. No other significant repeated sequences are present in the molecule. *b*, Comparison of the aligned ankyrin repeats. Shading indicates positions that are occupied by a single amino acid or two closely related amino acids in two-thirds or more of the repeats. The consensus sequence is shown below.

are: (1) residue 3 is much more polar in the ankyrin-like repeats than it is in erythroid ankyrin repeats; (2) the consensus Gly-His dipeptide at positions 13–14 in ankyrin repeats is not conserved in ankyrin-like repeats; (3) the consensus Asn/Asp at position 29 in the ankyrin repeats is less conserved in the ankyrin-like repeats; (4) the homologues favour Asp/Asn at the penultimate residue, whereas ankyrin repeats prefer threonine; (5) some of the ankyrin-like repeats (not shown in Fig. 4b) in each of the three invertebrate proteins have extra amino acids between or after putative helices 1 and 2.

The homologous invertebrate proteins are the products of the *Notch* (*Drosophila melanogaster*)⁴⁶, *lin-12* (*Caenorhabditis elegans*)⁴⁷ and *glp-1* (*C. elegans*)⁴⁸ genes. They are integral membrane proteins of similar structure: each has an external domain with several epidermal growth factor-like repeats, a single transmembrane segment and a cytoplasmic domain containing six ankyrin-like repeats. All three proteins regulate tissue differentiation. The *Notch* gene product is required for correct differentiation of neuroblasts from epidermoblasts (for a review, see ref. 43); but it is widely expressed in other embryonic and post-embryonic cells, particularly cells undergoing mitosis^{49–51,70}, and probably has other functions. The *lin-12* gene product controls the fate of certain cells in the ventral gonad of the nematode, and subsequently helps these cells direct other neighbouring cells to form the vulva^{52–55}. The *glp-1* gene product regulates the balance between germ-cell mitosis and meiosis in the nematode gonad^{48,56}. The structure of these three proteins and their involvement in fine pattern formation indicates that they are either receptors or adhesive proteins^{51,70}. We postulate that the invertebrate ankyrin-like repeats function as 'built-in' ankyrins and form binding sites for integral membrane proteins, tubulin or other proteins. This binding could regulate the

asymmetric re-distribution of the *Notch* protein within plasma membranes that occurs during *Drosophila* differentiation^{51,70}. Ankyrin-like repeats could also be the site of the genetically postulated association between *Notch* and one of the *Enhancer of split* gene products, an analogue of mammalian G-protein β -subunits⁵⁷ and a potential component of a *Notch* protein-linked signal-transduction complex.

The three yeast proteins are those encoded by *cdc10* (*Schizosaccharomyces pombe*)⁵⁸, *SW16* (*Saccharomyces cerevisiae*)⁵⁹ and *SW14* (*S. cerevisiae*)⁶⁰. Each contains two separated ankyrin-like repeats. The recently reported *SW14* gene product is not shown in Fig. 4, but its repeats are not significantly different from those of the other yeast proteins. All three proteins are involved in cell-cycle control. The *cdc10* gene product is one of two genes that are required for fission yeast to traverse 'start' and proceed from S to G1-phases of the cell cycle⁵⁹. Its biochemical function is unknown. The *SW14* and *SW16* gene products control production of the *HO*-encoded endonuclease that initiates the mating-type switch in fusion yeast⁶¹. Transcription of *HO* is restricted to a short period just after start and depends on a repeated element in the upstream region of the *HO* gene (the cell-cycle box, CCB) and on a factor, CCBF (cell-cycle box factor), that binds to the CCB elements. Formation of the CCBF-DNA complex requires *SW14* and *SW16*; *SW14*, at least, forms part of the complex⁶⁰ and must be located in the nucleus. It is not known whether the repeats function differently in nuclear and plasma membrane proteins, or whether they bind a common conserved protein or class of proteins that exists in both compartments.

It is notable, however, that at least five of the six proteins are involved with the cell cycle: *Notch* is primarily expressed in mitotic cells, *glp-1* regulates entry into mitosis versus meiosis,

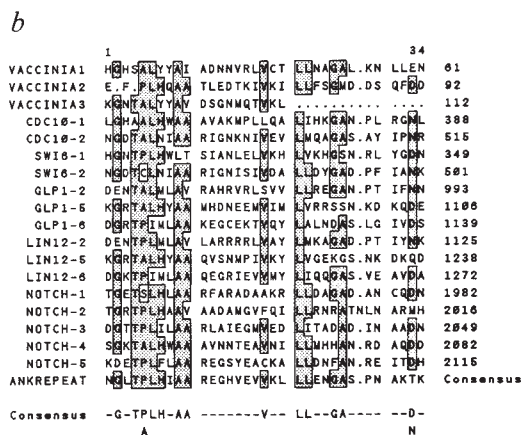
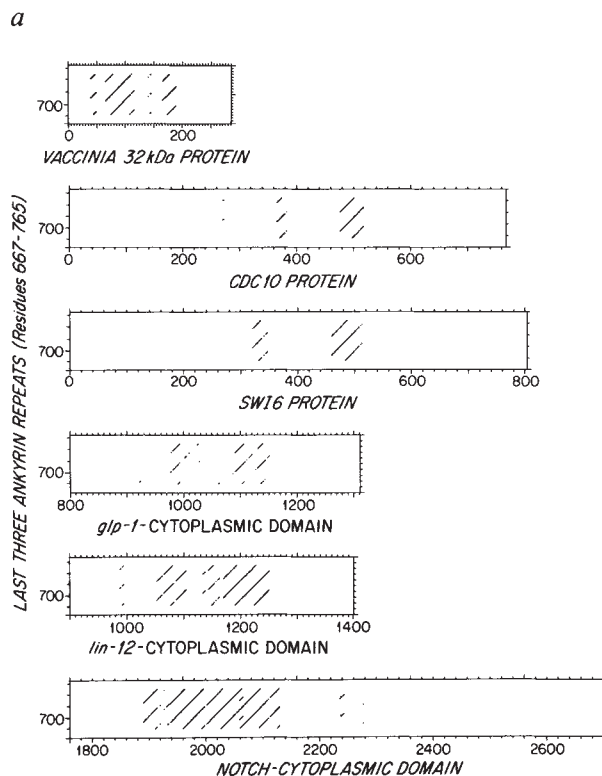


FIG. 4 Detection of ankyrin-related repeats in other proteins. *a*, Dot-matrix analyses of the last three ankyrin repeats (residues 667–765) versus: the vaccinia host-range protein⁶², two related yeast cell-cycle control proteins, the *cdc10* (*S. pombe*)⁵⁸ and *SW16* (*S. cerevisiae*)⁵⁹ gene products; and the

cytoplasmic domains of three proteins that regulate tissue differentiation, the *lin-12* (*C. elegans*)⁴⁷, *glp-1* (*C. elegans*)⁴⁸ and *Notch* (*D. melanogaster*)⁴⁶ gene products. The dot-matrix analyses were conducted using the high-stringency parameters described in Fig. 3. *b*, Comparison of the aligned ankyrin-related repeats with the complete erythrocyte ankyrin consensus sequence (ANKREPEAT). The location of the last residue of each repeat in its own protein sequence is listed on the right. The consensus sequence of the ankyrin-related repeats is at the bottom. Repeats 1, 3 and 4 of the *lin-12* and *glp-1* gene products and repeat 1 of the *Notch* gene product are not shown. They are longer, owing to insertions of various sizes in the middle and distal parts of the repeats (between or after putative helices 1 and 2).

METHODS. Using the UWGCG programs PROFILE and PROFILESEARCH, a weighted consensus sequence of the 22 aligned ankyrin repeats was derived and used to search the National Biomedical Research Foundation Protein Database (versions 20 and 21). Each of the 25 top-scoring proteins was then analysed with the program PROFILESEGMENTS and compared with the last three ankyrin repeats using the programs COMPARE and DOTPLOT.

cdc10 controls passage through cell-cycle start and commitment to mitosis, and *SW14/SW16*-induced transcription of the *HO* gene is limited to the 'post-start' period. The relationship of *lin-12* expression to the cell cycle has not been investigated.

The vaccinia⁶² (Fig. 4) and cowpox⁶³ (data not shown) host-range proteins contain two and one ankyrin-like repeats respectively. The single repeat in the cowpox virus (residues 458–490; ref. 63) is 39% identical to the ANKREPEAT consensus sequence (Fig. 4b). The viral proteins determine infectivity of their hosts, but, unfortunately, nothing is known about their cellular location or biochemical mechanism.

Finally, six or seven ankyrin-like repeats have recently been discovered in *bcl-3*, a candidate proto-oncogene on chromosome 19 that is activated by translocation to the 5' side of the IgH gene in some patients with B-cell chronic lymphocytic leukaemia (H. Ohno, G. Takimoto and T. W. McKeithan, personal communication). This observation strengthens the inference that ankyrin-like repeats are involved in cell growth and differentiation.

Discussion

In summary, ankyrins constitute a new family of proteins that seem to function as 'molecular brokers'; that is, they coordinate interactions between various integral membrane proteins and cytoskeletal elements. The studies described here define the structural domains of erythrocyte ankyrin, the first-described member of the family, and identify a cluster of characteristic repeats also present in slightly altered form in three invertebrate proteins regulating tissue differentiation, three yeast proteins having functions related to the cell cycle, a putative human proto-oncogene, and the host-range proteins of two viruses. Structural-prediction algorithms indicate a helix-turn-helix motif with amphipathic helices for these repeats. We postulate that the ankyrin repeats are arranged in an isotropic array, and

that different repeats, singly or in combination, form binding sites for several integral membrane proteins and tubulin. It should be possible to test these hypotheses and identify other binding sites using expressed segments of erythrocyte ankyrin. The availability of erythrocyte ankyrin clones will also facilitate cloning of other ankyrins, analysis of ankyrin genomic structure, and detection of ankyrin defects in red cells and other tissues. Among the proteins containing ankyrin or ankyrin-like repeats, only ankyrin is available in amounts suitable for structural analysis and binding studies. Also, ankyrin and the red-cell membrane are very well understood compared with the ankyrin homologues and the membranes of the cells containing them. The simple discovery that the cytoplasmic repeats of the *Notch*, *lin-12* and *glp-1* gene products are related to ankyrin limits their possible functions, and the evolutionary conservation of these repeats emphasizes their importance. This knowledge and future discoveries about the structure and function of ankyrin should be useful in deciphering how ankyrin homologues function in tissue differentiation and cell-cycle control.

Note added in proof: In parallel experiments S. Lambert *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, in the press) have also determined the sequence of human erythrocyte ankyrin. They find alternative sequences at the C terminus that probably result from differential splicing: (1) deletion of bp 5,630–5,704, amino acids 1,849–1,873 (our numbering) in one clone and (2) insertion of 135 bp in this gap in a second clone. The latter change replaces the 33 acidic C-terminal amino acids of the present sequence with a highly basic 32 amino-acid sequence. They also find: (1) a CAG sequence at bp 771–773 (our numbering), which would change residue 230 from Ala to Ser; (2) an A at bp 4,720, which would change amino acid 1,546 from Val to Ile; and (3) the silent substitutions of C for T at bp 2,157, C for G at bp 3,030, and GG for AA at bp 6,098–6,099. □

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Galaxy mass deduced from the structure of Einstein ring MG1654+1346

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ELLIPTICAL galaxies acting as gravitational lenses occasionally produce spectacular images—Einstein rings—of distant objects. Giant arcs¹ and radio rings² have been observed. A wide variety of image morphologies is possible, the generation of which is qualitatively understood in terms of large magnifications at caustic and critical lines in the lensing geometry^{3–5}. From the angular size of the image, and with knowledge of the distances of the lensed and lensing object, rough estimates of the mass of the lensing galaxy can be obtained. We have made high-resolution radio-interferometric observations of MG1654+1346 (ref. 6) a radio quasar in near-perfect alignment with an elliptical galaxy, and show that radio emission is distorted into a narrow Einstein ring that lies within a diamond-shaped region bounded by caustic lines. From the details of the ring structure, a new model for the lensing geometry is deduced which leads to a more accurate estimate of the mass of the lensing galaxy.

A unique feature of the gravitational lens system MG1654+1346 is the simplicity of the lens, a single relatively bright galaxy. This simple configuration allows a straightforward test of models of galactic mass. To test the gravitational-lens models we carried out 3 hours of 8.4-GHz observations of MG1654+1346 with the National Radio Astronomy Observatory's Very Large Array (VLA) telescope; a full report of this data will be published elsewhere. Figure 1 presents the 8.4-GHz total-intensity map of MG1654+1346 superimposed on a grey-scale plot of an R-band optical image obtained with the 1.3-m McGraw-Hill telescope⁶. The grey-scale image shows a faint, distant (redshift $z=1.75$) quasar north of an extended galaxy. The nearer, low-redshift ($z=0.254$) galaxy is brighter than the quasar at optical wavelengths but the galaxy is not detected at radio wavelengths. The contours mark the structure of radio emission of a typical double-lobed quasar; such objects^{7,8} have two symmetrical radio lobes lying on opposite sides of the optically detected quasar core. Figure 1 shows that the southern lobe falls directly behind the galaxy and is distorted into an Einstein ring. The ring is roughly circularly symmetric with two bright semicircular components north and south of the galaxy.

The central cores of quasars are generally detected at both radio and optical wavelengths. Although the core of MG1654+1346 was not detected at radio wavelengths in the earlier VLA observations⁶, our model of the radio source predicted that the quasar core would be detected in more-sensitive observations. This is confirmed in Fig. 1; the quasar core is detected at right ascension $16^{\text{h}} 52^{\text{m}} 23.89^{\text{s}}$ and declination $13^{\circ} 51' 9.83''$ (B1950). As is typical of quasars, both lobes emit strongly at radio wavelengths, but not at optical wavelengths. Radio lobes show a variety of structures, but generally display diffuse emission with compact features, called hotspots, at the edges most distant from the core. The hotspots such as those in MG1654+1346 often emit strongly polarized radio flux. Diffuse radio emission from the ring trails back towards the optically detected quasar in a manner similar to that of the northern lobe.

A distinguishing feature of gravitational lensing is that it is not dispersive; each image is formed from light at all wavelengths travelling a single path. This implies that all images of a single source have identical spectra and polarization. The polarization properties of MG1654+1346 are particularly important for determining the source structure. The brightest features of the ring are displayed in Fig. 2, a contour plot of the polarized flux density distribution superimposed on a grey-scale plot of the total intensity distribution at 8.4 GHz. Peak total intensity features are marked in Fig. 2 and indicate that the source is composed of three components: a weakly polarized hotspot, a strongly polarized hotspot and diffuse emission with structure similar to the northern lobe. The diffuse emission is required to produce the faint structure of the ring seen in Fig. 1. The images of each hotspot have similar polarized flux orientation angles, as is required. The pairs of images of the weakly and strongly polarized hotspots are separated by $1.89 \pm 0.05''$ and $2.04 \pm 0.05''$, respectively.

The galaxy mass lying in the ring is calculated from the size of the ring. The radius of the Einstein ring θ_r in radians for a spherically symmetric distribution of mass M_r is

$$\theta_r = \left(\frac{4G}{c^2} \frac{D_{ls}}{D_s} \frac{M_r}{D_l} \right)^{1/2}$$

where D_l , D_s and D_{ls} are, respectively, the distances to the lens and source, and the distance between the lens and the source⁹. All distances and constants are expressed in m.k.s. units, G is Newton's constant and c is the speed of light. We note that the lens is much closer to the observer than it is to MG1654+1346, making the ratio D_{ls}/D_s near unity and largely independent of

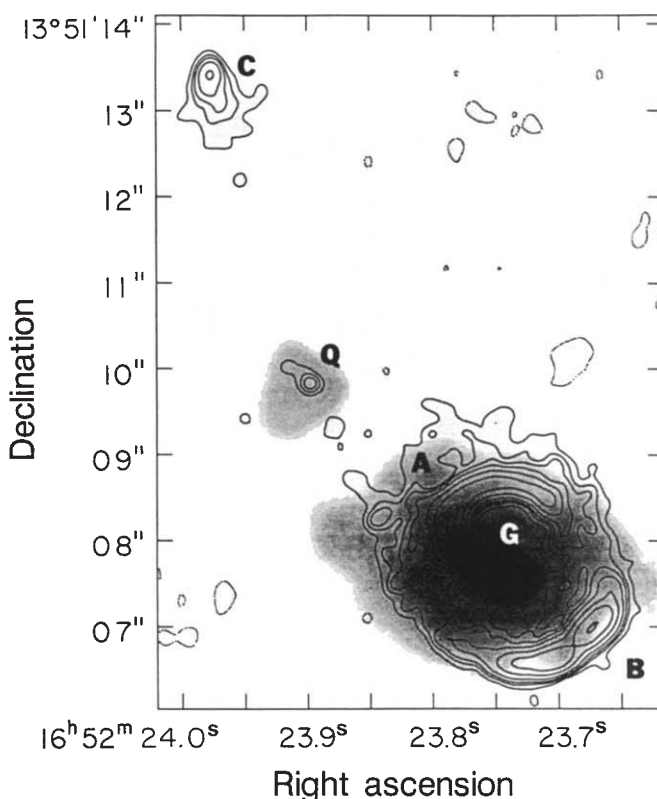


FIG. 1 Contour plot of 8.4-GHz radio image of MG1654+1346 superimposed on an R-band optical image of the quasar Q and galaxy G. The radio image was constructed from VLA observations in A and B configurations. The contour levels are $-0.1, 0.1, 0.2, 0.3, 0.5, 1, 2$ and 4 mJy. The restoring beam has a $0.2''$ circular FWHM (full width at half maximum) gaussian profile. The angular resolution of the optical image is $\sim 2''$. The Einstein ring is composed of two semicircular components labelled A and B. The northern lobe of the quasar is labelled C.

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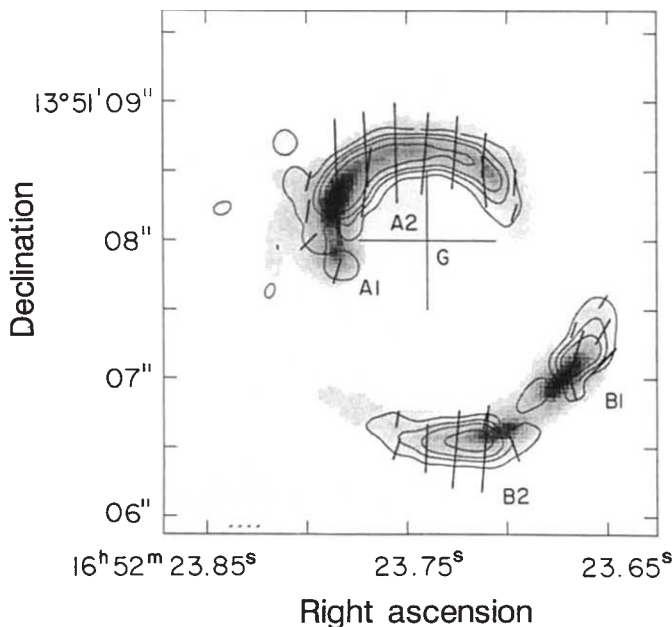


FIG. 2 Contour plot of 8.4-GHz linearly polarized radio emission of MG1654 + 1346 superimposed on a total-intensity grey-scale plot. The contour levels are $-0.1, 0.1, 0.2, 0.3, 0.5$ and 1.0 mJy. The orientation of the polarized flux is indicated by line segments. The pair of weakly polarized peaks of the total-intensity map are labelled A_1 and B_1 . The pair of peaks A_2 and B_2 are images of a different, more strongly polarized component. The faint peak A_1 is just south of the much brighter peak A_2 , which is extended east-west. Components B_1 and B_2 have similar brightness and size. The large cross indicates the location of the centre of the optically detected galaxy, G.

cosmological parameters. The ratio of the mass of the galaxy and the distance to the lens enters as a single factor that determines the Einstein ring size. The Einstein ring radius is $0.98 \pm 0.02''$, measured from the average of the hotspot separations. The resulting mass estimate is $M_r = 8.98 \pm 0.37 \times 10^{10} h^{-1} M_\odot$, assuming a filled-beam cosmology⁹, $q_0 = 0.5$, $H_0 = 100 h \text{ km s}^{-1} \text{ Mpc}^{-1}$, and where h is a factor that accommodates the dependence of the estimates on the value of H_0 . The systematic uncertainty associated with the circular-symmetry approximation is not included in the error estimate.

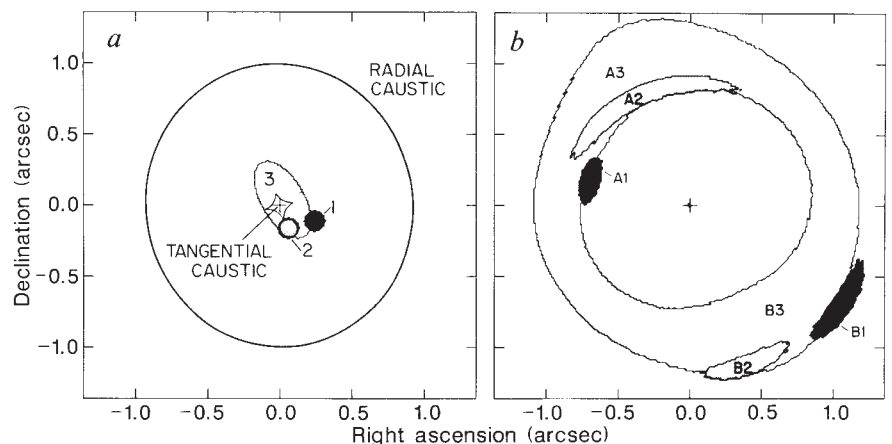
We present a model for the southern lobe's structure combined with an elliptical galaxy model to reproduce the features of the Einstein ring. Bourassa and Kantowski³ were the first to present a formalism that explained the multiplicity of images in terms of caustic and critical lines. These models describe the number of images and their orientation in terms of the source location within radial and/or tangential caustic lines of high image magnification. These lines mark the regions where a single source

component is magnified and distorted into one, three or five images. Blandford⁴ and Narayan⁵ have shown that sources lying near the outer caustic are radially distorted along the line connecting the source and centre of the lens. Sources lying near the inner, diamond-shaped, caustic are tangentially distorted along a circle surrounding the centre of the lens.

The size and orientation of the caustics are dependent on the mass distribution of the lensing galaxy. We use a modified version of the galaxy model of Kochanek *et al.*¹⁰ with two free parameters, the mass inside the ring and the eccentricity of the projected mass distribution ϵ . Our model corresponds to a galaxy without a massive core. A small value of eccentricity, $\epsilon = 0.05$, produced the qualitative features of MG1654 + 1346. A satisfactory model was found by visual inspection of images produced by galaxy models with eccentricity range $0 \leq \epsilon \leq 0.5$. A variety of plausible source models and positions was tested with each galaxy model. The galaxy model potential is determined by the ring size and corresponds to a one-dimensional velocity dispersion of $216 \pm 5 \text{ km s}^{-1}$. The position and orientation of the galaxy define the coordinate system and are not free parameters. The size of the diamond caustic increases with increasing eccentricity of the galaxy. For a spherically symmetric galaxy, the diamond caustic is a single point.

Components of MG1654 + 1346 lie in all regions defined by the caustics. Figure 1 shows only single images of the northern lobe and quasar core because these components lie outside the radial caustic. The southern lobe is assumed to look like the northern lobe and the polarization structure requires three model components, labelled 1, 2 and 3 in Fig. 3a. The caustic lines are drawn relative to the centre of the galaxy acting as lens. Hotspot 1 lies outside the diamond caustic, but inside the three-image region. Hotspot 2 lies on the diamond caustic, closer to the centre of the galaxy than component 1. Component 3, the diffuse emission, is faint but covers a large region including all of the diamond caustic. Figure 3b shows the results of gravitational-lens imaging of the source by the galaxy. The figure shows three images of each of the hotspots: two bright images north and south of the galaxy, and a faint image near the centre of the galaxy. The image of the diffuse emission forms a complete ring. Images of component 1 show the properties typical of a nearly circularly symmetric lens; the southern image lies on the same side of the galaxy as the original source position and is therefore brighter and further from the galaxy than the northern image. Because source component 2 lies on the diamond caustic, the image on the north side of the lens is greatly magnified and bent. The model result in Fig. 3b should be compared with the observed emission from the ring shown in Fig. 2. The images near the centre of the ring are too faint to be detected in these observations. The angular separation between the model hotspots is only $\sim 0.2''$, but the hotspots are resolved by these observations because the source is magnified by gravitational

FIG. 3 a, Contour plot of the lens source plane showing three components of the southern-lobe model. These components lie entirely within the outer radial caustic and partially within the tangential (diamond) caustic. The hotspots are labelled 1 and 2 and the diffuse emission is labelled 3. b, Contour plot of the image plane, showing the locations of images of the three components of the source model. The bright images of the hotspots are distorted tangentially because the original components lie near the tangential caustic. The diffuse emission from the lobe is magnified and distorted into a complete Einstein ring.



lensing. The model magnification increases the observed source flux density by a factor of ~ 14 . The model for MG1654+1346 does not place the two lobes and core exactly on a line, but this type of structure is exhibited by several quasars found in the Lawrence *et al.*⁸ survey.

The mass of the galaxy placed symmetrically outside the images does not affect the image separation. Therefore image separation measures only the mass lying between the images, and a galaxy model must be used to estimate the total galaxy mass. We estimate the mass outside the images by assuming the projected mass-to-light ratio is the same inside and outside the ring. The ratio of the light inside the ring to the total galaxy emission is 0.302 ± 0.03 measured from a Gunn *r* band image (D. Schneider, personal communication). The total mass estimate for the galaxy is $M_g \sim M_r / (0.302 \pm 0.03) = 2.97 \pm 0.32 \times 10^{11} h^{-1} M_\odot$. This is a lower estimate to the galaxy mass because observations indicate that the mass-to-light ratio increases with increasing distance from the centre of galaxies. The estimated total B-band luminosity of the galaxy⁶ is $1.87 \pm 0.18 \times 10^{10} h^{-2} L_\odot$, where $L_\odot$ is the luminosity of the Sun. Scaling the total luminosity by the measured fraction of light originating in the ring yields a mass-to-light ratio M_r/L_r of $15.9 \pm 2.3 h$. This estimate of the mass combined with direct measurement of the optical luminosity measures the amount of dark matter lying in the central region of the galaxy.

The higher-resolution image and new polarization map of MG1654+1346 strongly support the gravitational-lens model. The new model shows that the brightest components on opposite sides of the ring are not images of a single component, as was suggested by earlier observations. The mass of the lensing galaxy is well determined by measurement of each pair of image separations, but iterative approaches to modelling, such as that of Kochanek *et al.*¹⁰, should also be applied to this system. Reliable mass measurements of distant galaxies are an important test of our understanding of cosmology. $\square$

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Are some BL Lacs artefacts of gravitational lensing?

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WE suggested in 1985 that a significant fraction of BL Lacertae objects, a kind of lineless quasar, seen in nearby galaxies are in fact images, gravitationally lensed and substantially amplified by stars in the nearby galaxy, of background objects, optically violent variable (OVV) quasars at redshifts $z > 1$ (ref. 1). This hypothesis was made on the basis of certain general similarities between BL Lacs and OVVs, but for two recently observed BL Lacs^{2,3} a strong case can be made that the accompanying elliptical galaxy

is a foreground object. In addition, we argue that the distribution of BL Lac redshifts is hard to understand without gravitational lensing, unless we happen to be at a very local maximum of the spatial cosmic distribution of BL Lacs. Our analysis also indicates that the galaxies whose stars are likely to act as microlenses will be found in two peaks, one nearby, with redshift 0.05–0.10, and the other near the distant quasar.

There are two reasons for thinking that microlensing by stars might be important for understanding BL Lac objects. Blazars or optically violent variables seen at normal quasar redshifts have strong variable polarized continua and show rapid temporal variations, indicating a small angular size. Ordinary stars along the line of sight should therefore significantly focus and amplify the continuum high-energy radiation. The line-producing regions are far too big to be lensed, so that amplified objects will have relatively weak emission lines but otherwise will be OVVs. Such objects, the existence of which can be theoretically predicted with some confidence, will be seen in projection against normal galaxies and have, in general, just those properties that we associate with BL Lac objects (with the rapid variability intrinsic to the OVV and not caused by microlensing). This led us to propose¹ that a significant number of the BL Lac objects seen in nearby galaxies are not truly in them but merely projected on them and magnified in brightness by stellar lensing. It remains to be seen whether one can predict the total number correctly and, more specifically, understand the number-magnitude relation, the redshift distribution, and the type, magnitude and redshift distribution of the lensing galaxies.

The object 0846+51W1 (ref. 4) had, when bright, all of the attributes of a BL Lac but at minimum light was a normal quasar with redshift 1.860. It is 12" away from a foreground galaxy of size comparable to the separation and is quite red. There is also a still closer ($\sim 2''$) red nebulosity consistent with a foreground elliptical galaxy. The statistical association is unlikely, *a priori*. The observations are those expected if a star in one of the foreground objects crossed in front of the central part of the background quasar, with some of the anomalous redness of the spectra caused by galactic obscuration in the intervening galaxy. The rise time of months is shorter than that predicted for optically thin ($\tau \ll 1$) lenses⁵ but for τ approaching (but not reaching) unity, shorter fluctuation times are expected^{6,7}.

The weak emission lines of object 0215+15 indicate a redshift of 1.715 (ref. 8). But a very rich absorption-line system at smaller redshift (1.345) shows Zn II (unique among quasars), indicating cool gas in a normal galaxy along the line of sight. "Indeed, judging by the strength and complexity of the lines, the intercept probably passes through the inner region of the intervening system", (ref. 8) at too large a redshift to be detected as an image.

In 0537–441 (refs 2, 3) the emission-line redshift is 0.894. The BL Lac appears to lie at the centre of a nearby elliptical galaxy of unmeasured redshift but with a large angular size that makes a redshift of 0.9 quite improbable. A companion elliptical galaxy 12" away (comparable in distance to the image size) has a redshift of 0.186. Thus it is likely that the 'parent' elliptical galaxy will be found to have a redshift of ~ 0.19 , much smaller than that of the BL Lac. The most striking case is 0235+164 (refs 2, 3) where the BL Lac has an emission-line redshift of 0.94. It is offset by a measurable amount (but $< 1''$) from the centre of an elliptical galaxy with a stellar redshift of 0.524, which is in a small group of galaxies. There is also an emission-line redshift of 0.525 from O II and H lines which, had the background BL Lac emission lines at $z = 0.94$ not been seen, would have been taken as proof that the BL Lac was indeed in the foreground galaxy.

The statistical properties of the observed BL Lacs are very strange. The tabulation by Burbidge and Hewitt⁹ contains 31 objects and their redshifts, of which eight have identified emission lines. Three of these eight are in the above list of objects for which there is strong evidence of a foreground galaxy

TABLE 1 Redshifts and magnitudes of putative BL Lacs

Group	Redshift type	Number of objects	$\bar{z}$	$\bar{m}$
A ₁	z_{gal}	10	0.049 ± 0.015	14.73 ± 0.75
A ₂	z_{gal}	11	0.243 ± 0.141	17.3 ± 1.8
B	$z_{\text{ab}}, z_{\text{em}}$	10	1.047 ± 0.565	17.4 ± 1.8

between the BL Lac and Earth (the fourth case 0537-441 is not listed in ref. 9). The tabulation can be divided into two groups. The ten identified by either emission or absorption redshifts we call Group B (these comprise groups (a) and (b) of ref. 9). Group A contains 21 objects, the redshifts of which were determined by the galaxy in which the BL Lac apparently resides. To make the groups more even in size, we subdivide Group A at the median redshift into the 10 objects with the smallest redshifts, A₁, and the remaining 11 objects, A₂. The properties are summarized in Table 1.

Consider sample A₁, the nearest group, with $\bar{z} = 0.05$. If we assume local cosmological uniformity, then in a sphere with mean redshift of 0.15 and a relative radial distribution similar to Group A₁, we have a sampling volume 27 times larger and expect to find 270 ± 80 objects. These would have a mean magnitude $\bar{m}$, owing to the distance increase of a factor of three, of 17.1. Thus they are the same apparent brightness as Group A₂ and should be no more difficult to find than those in A₂. Where are they? It seems that Group A₂ is incomplete by a factor of 25. But it is worse than this, because the volume surveyed in A₂ is in fact (from the redshift ratio) 120 times larger rather than 27 times larger. It seems that we live at the sharp peak of a large local density concentration of BL Lacs. The overdensity of galaxies in the local supercluster is only about three, so Group A₂ is observationally incomplete by over a factor of ten for objects of the same apparent magnitude (a relatively bright $m \approx 17$) as the nearby ones.

The typical magnitude and redshift of Group B objects are similar to those in comparable quasar surveys; the Bracesi survey¹⁰ of ultraviolet bright quasars has $\bar{m} = 17.3$ and $\bar{z} = 1.01$. From this, we estimate that a complete all-sky magnitude-limited sample of quasars with the same $\bar{m}$ as Group B would contain 10^5 objects. Thus the ratio of quasars to BL Lacs is $10^4 f_B : 1$ where f_B is the incompleteness factor for Group B. We would therefore expect $10^{-4}/f_B$ BL Lacs as close or closer than the closest quasar 3C273, and brighter than $m \approx 17$. In fact there are $>10^5$ times more of them observed, and we know that the nearby sample is seriously incomplete. Thus Group B would have to be larger by a factor of 10^5 for the two sets of objects to have comparable space distributions. As others have noted, this huge difference can be understood if the strong positive evolution of the normal quasars is inverted for the BL Lacs. Plausible corrections to allow for the effects of colours on apparent evolution (K correction) could alter this conclusion. This anomaly is surprising but poses no theoretical difficulties given our almost complete ignorance of the phenomena producing BL Lacs. Finally, the *a priori* probabilities of the coincidence on the sky between the objects in Group B and relatively bright foreground objects are difficult to estimate, but the probability of finding two of eight randomly placed objects within $2''$ of a galaxy brighter than $m = 20$ is $<10^{-6}$.

Can gravitational lensing explain these perplexing observations? The redshift distribution expected of lenses was calculated for point-mass galaxies¹¹ (that is, galaxies represented as singular isothermal spheres). We found a broad distribution peaking near $z = 0.5-1.0$, but with a very low probability that the lens would be either near the observer or near the quasar. Another prediction made was that, because the lensing cross-section is proportional to the fourth power of the internal velocity dispersion of the lensing galaxy, elliptical galaxies should be greatly over-represented in the sample. Both predic-

tions are in accord with the handful of good candidates for gravitationally lensed quasars. We have calculated^{12,13} the expected magnitude distribution of strongly amplified objects and predicted a flattening at bright magnitudes in accord with the observations. Thus a general agreement with the lensing hypothesis is apparent, but there is one serious discrepancy.

It seems almost as difficult for the lensing hypothesis to account for the large number of small z sources as it is for more conventional models. An important clue is provided by Huchra's object¹⁴, a multiply imaged quasar of apparent magnitude 18 seen projected on the core of a nearby bright Shapley Ames galaxy. In the absence of amplification bias, the *a priori* probability of having one such system in the sky is estimated¹⁵ to be 10^{-4} . Amplification in brightness has brought an otherwise faint quasar up to an easily detectable level. As the quasar is found in the core of the galaxy, the calculations in ref. 11 do not apply, because the typically modelled galaxies have infinite central surface brightness, that is, no core.

If galaxies are treated in the other limit, as simple disks of fixed surface density, what would then be expected? First we specify the disk surface density Σ in units of the cosmological surface density $\Sigma_0 \equiv cH_0/2\pi G \approx 0.23 \text{ h g cm}^{-2}$. A critical density Σ_{crit} can be defined for a sheet of matter¹¹ at redshift $x \equiv 1 + z_{\text{gal}}$ such that a quasar at $y \equiv 1 + z_q$ seen through the sheet would have, formally, infinite brightness, because all rays from the quasar would converge on the observer. For the empty-beam approximation in a universe with a cosmological parameter Ω of unity we have $\Sigma_{\text{crit}} = (5/4)\Sigma_0 x^4 (y^{5/2} - 1) / [(y^{5/2} - x^{5/2}) \cdot (x^{5/2} - 1)]$. For fixed quasar redshift y , this function has a minimum at intermediate redshifts but the cross-section becomes large for $z \rightarrow 0$ and $z \rightarrow z_q$. Thus, if the hypothetical disk galaxy considered has surface density $\Sigma_{\text{gal}} \gg \Sigma_0$, then for intermediate redshifts there are three images, two amplified ones that pass on either side of the disk (which for these images acts like a point mass), and one that passes through the disk which is demagnified because the amplification is $A = (1 - \Sigma_{\text{gal}}/\Sigma_{\text{crit}})^{-2}$. But at small and large redshifts, where $\Sigma \approx \Sigma_{\text{crit}}$, large amplification is possible for the central image which passes through the disk and can be further amplified by microlensing. Microlensing provides no net amplification, but it does produce more bright images which will then be found in magnitude-limited surveys¹⁶. Exactly at $\Sigma_{\text{gal}} = \Sigma_{\text{crit}}$, however, the cross-section (the probability of lensing) can be shown to go to zero. So we expect a maximum effect near but not at $\Sigma_{\text{gal}} = \Sigma_{\text{crit}}$. Figure 1 shows the effect. We take a representative disk with

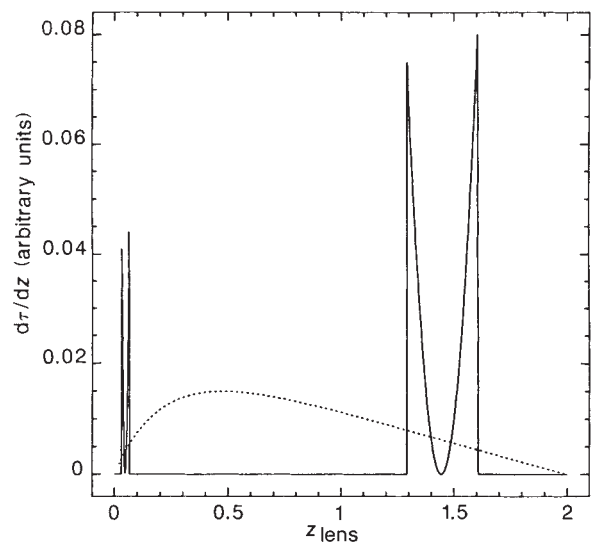


FIG. 1 Expected distribution in redshift of the lensing galaxy for a quasar at $z=2$ ($h=\Omega=1$, empty beam assumed). Dotted line shows distribution for point masses¹¹, solid for disks with same mean Ω and surface density 2 g cm^{-2} .

$\Sigma_0 = 2 \text{ h g cm}^{-2}$ and show the distribution in redshift of expected lens positions assuming that the lensed quasar is at $z=2$ and minimum amplification 10. There are two peaks at small redshift near $z \approx 0.05$ and two peaks near redshift 1.6, with no large amplification lensing between these regions. By contrast, the dotted line shows the expected distribution for point masses¹¹ with a peak near redshift 0.5. Mini-lensing, not included in Fig. 1, would increase the effect, because $\tau \approx 1$ in the peaks. The BL Lac effect is due to mini-lensing, which is most probable in the central regions of the lensing galaxy. Amplification bias due to the combined effect of the lensing by the smooth galactic potential well and by mini-lensing, however, makes us select as BL Lacs exactly those objects near to the two critical redshifts observed in Fig. 1. If the angular size of the parent quasar is large it cannot be mini-lensed but, like Huchra's objects, still will be seen in one of the peaks.

This new theoretical argument indicates that mini-lensing should typically occur quite near the observer, at small redshifts. Combining this with statistical evidence strengthens the case that some BL Lacs are actually distant OVV quasars lensed by intervening galaxies, although a more detailed statistical analysis is still required¹⁷. The best way to test this hypothesis, of course, is observational, to see if the BL Lac has any absorption or emission-line redshift larger than the galaxy in which it apparently resides. $\square$

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Global atmospheric chemistry of CFC-123

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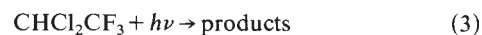
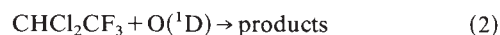
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THE compound 1,1-dichloro-2,2,2-trifluoroethane (CFC-123), which is potentially usable as a foam-blowing agent in the plastics industry, an aerosol propellant and a refrigerant, has been proposed as an industrial substitute for trichlorofluoromethane (CFC-11), the use of which is increasingly restricted because of its effects on the ozone layer and on climate^{1–3}. It is expected that CFC-123, although like CFC-11 an absorber of infrared radiation, will be less stable in the atmosphere because of its expected reaction with OH radicals in the troposphere. Using a three-dimensional global model of the atmosphere, we have calculated the chemical destruction rates of CFC-123 by various processes, confirming that the chief sink is destruction by OH radicals below 12 km, which accounts for 88% of its loss. The calculated destruction rate is greatest in the equatorial region below 2 km. The calculated steady-state lifetime of CFC-123 is 1.5 years, based on the best available

estimate of the rate constant of the reaction with OH. This lifetime is very much shorter than that of CFC-11, the destruction of which is largely confined to the stratosphere. For equal rates (by mass) of CFC-123 and CFC-11 emission to the atmosphere, the molar content in the atmosphere and the injection rate of chlorine into the stratosphere are, respectively, 48 and 14 times greater for CFC-11 than for CFC-123 in steady-state.

The atmospheric concentrations of chlorofluorocarbons such as CFC-123 depend on their rates of industrial emission, photochemical destruction and atmospheric circulation. These rates vary substantially over the globe and cannot realistically be included in one-dimensional models. Multi-dimensional models are required for this purpose and we use specifically a three-dimensional model.

Our three-dimensional model of global atmospheric circulation and chemistry solves the relevant three-dimensional continuity equations using global circulation and ozone data calculated using the same model^{4,5}. This model has 26 levels between altitudes of 0 and 72 km, with variables at each level expressed in terms of 79 spherical harmonics (rhomboidal truncation, zonal wavenumbers 0–6). The chemical destruction rates are calculated on a 15°-latitude by 16°-longitude grid. The equations are solved in wave-vector space with grid-to-spectral transformations being accomplished using fast Fourier transforms. 1,1-dichloro-2,2,2-trifluoroethane is destroyed by the reactions



with rate constants for reactions 1 and 2 being $k_1 = 6.4 \times 10^{-13} \exp(-850/T) \text{ cm}^3 \text{ s}^{-1}$ and $k_2 = 2.2 \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$ (ref. 6). Absorption cross-sections for the ultraviolet photons in reaction 3 are temperature dependent and we use the most recent recommendations⁶. The concentrations of O(¹D) atoms and the photodissociation rates are calculated at each time step whereas the zonally averaged seasonally varying concentrations of OH radicals are specified in the model^{4,5}. The tropospheric average OH radical concentration is $(8.6\text{--}9.5) \times 10^5 \text{ cm}^{-3}$ and the OH radical concentrations have been tested by demanding agreement between the lifetime of CH_3CCl_3 predicted in our three-dimensional model and the 6.3-yr lifetime recently deduced from observations⁷. For the ultraviolet absorption cross-section we use the present best estimates⁸ for the absorption of O₂ in the Herzberg continuum region.

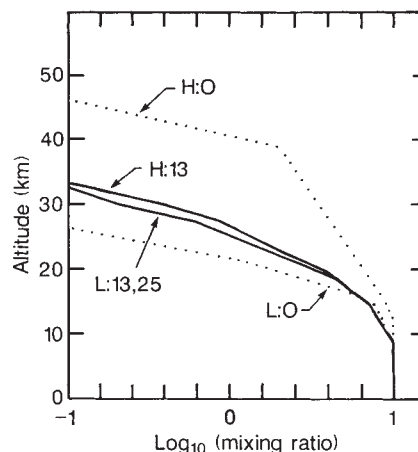


FIG. 1 Horizontal average mixing ratios (p.p.t.v.) as a function of altitude in the model runs H and L. Altitude profiles are given at the beginning and after integration for 13 and 25 model months, at which time the profiles were essentially constant.

To achieve a steady state for total CFC-123 atmospheric

As shown in Fig. 1, the predicted vertical distributions for

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As shown in Fig. 4, destruction of CEC-123 is due in largest

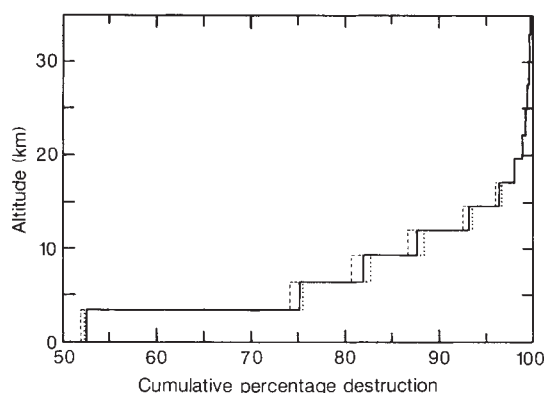


FIG. 5 Cumulative percentage destruction occurring below each model level at the end of model months 21 (December, dotted line), 24 (March, solid line) and 27 (June, dotted line) in run L. Almost identical results are obtained for the H run.

function of model altitude is shown in Fig. 5. Destruction is heavily confined to the troposphere, with 88% occurring below 12 km, in approximate agreement with independent one-dimensional model calculations¹¹.

The ratio R_1 of the steady-state rates of injection of chlorine into the stratosphere by CFC-123 and CFC-11, for equal mass emission rates, is a measure of the relative potential capability for ozone depletion by these species and is given by

$$R_1 = \frac{f(\text{CFC-123}) \times n(\text{CFC-123}) \times m(\text{CFC-11})}{f(\text{CFC-11}) \times n(\text{CFC-11}) \times m(\text{CFC-123})} = 0.072 \quad (4)$$

where f is the fraction of total destruction occurring in the stratosphere (0.12 for CFC-123 from our calculations and 1.0 for CFC-11 (ref. 4)), n is the number of chlorine atoms per molecule, and m is the relative molecular mass. Also, because CFC-123 and CFC-11 have similar infrared absorption properties (J. Elkins and K. Gurney, personal communication) the ratio R_2 of the steady-state global molar contents of CFC-123 and CFC-11 for equal mass emission rates is an approximate measure of their relative potential contribution to the greenhouse effect and is given by

$$R_2 = \frac{\tau(\text{CFC-123}) \times m(\text{CFC-11})}{\tau(\text{CFC-11}) \times m(\text{CFC-123})} = 0.021 \quad (5)$$

where $\tau(\text{CFC-123}) = 1.52$ yr from our work and the steady-state lifetime of CFC-11 is taken as $\tau(\text{CFC-11}) = 65$ yr (refs 4, 13). Evidently the potential influence of CFC-123 on the ozone layer and climate is much smaller than that of CFC-11, provided that emissions of the two compounds are comparable. □

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A novel high-efficiency crystal/polymer composite material for nonlinear optics

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FOR practical applications in optoelectronic devices, nonlinear optical materials should ideally combine appropriate optical properties (that is, a nonlinear response to an electric field, characterized by second-harmonic generation) with the mechanical properties, such as strength and rigidity, required for ease of processability. As reported here, we have developed a new class of material that combines these attributes, by growing aligned crystals of an optically nonlinear organic compound in a transparent polymer matrix. The host conveys desirable mechanical characteristics to the otherwise fragile organic crystals. The composites are transparent and non-scattering, with a refractive index that can be varied by modification of the polymer host. Given, in addition, the high chemical stability of these materials, we believe that they will have an important part to play in the development of optoelectronic devices.

Attempts to achieve the combination of desirable properties described above are not new. The method described here is based on the temperature-gradient zone-melting (TGZM) technique, used widely in the preparation of solids for special purposes^{1,2}. Materials analogous to our composites are found in nature³ (for example, bone, teeth and shells), and examination of crystal growth in such composites indicates that the desirable qualities of the guest species are strongly enhanced if the guest microcrystals are aligned within the polymeric host. Methods such as polymer film stretching^{4,5} and electric-field poling^{6,7} have been devised to achieve some degree of alignment in synthetic composites, but have met with limited success.

Here we use the TGZM method to grow aligned crystals of 3-nitroaniline (mNA) in transparent matrices of poly(methyl methacrylate) (PMMA) and poly(vinylcarbazole) (PVK). The thermal-gradient apparatus consists of two aluminium blocks separated by a 1-mm thermally insulating layer. One block is heated and the other is cooled to create a sharp thermal gradient.

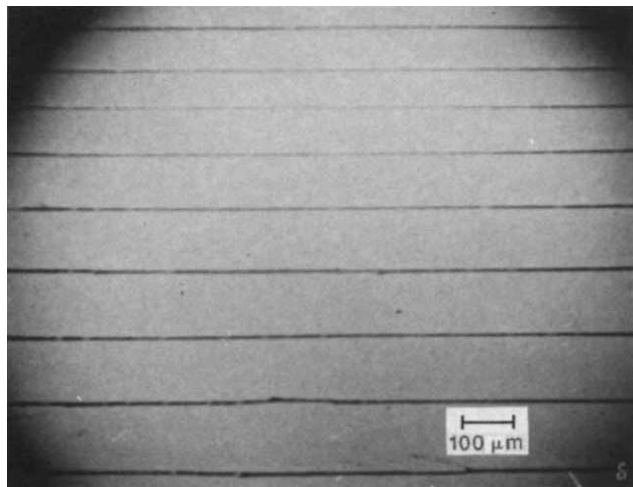


FIG. 1 An mNA-PMMA composite containing highly aligned crystals, taken through a polarizing microscope.

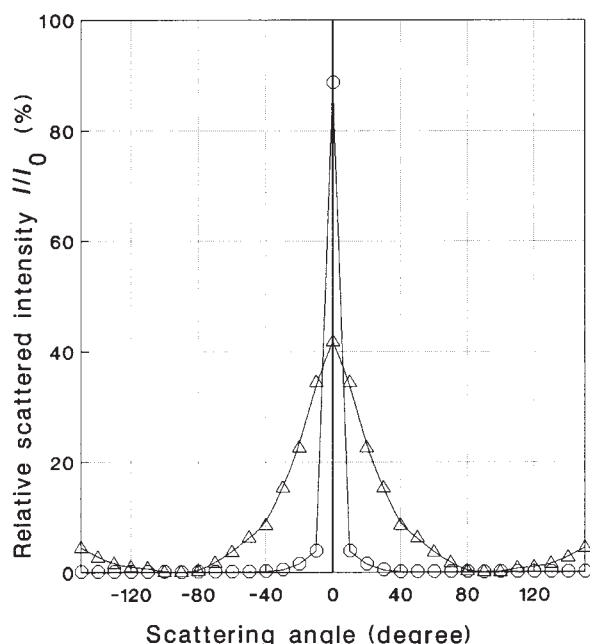


FIG. 2 The angular distribution of scattered 632-nm He-Ne laser light from partially aligned (Δ) and well aligned ($\circ$) *mNA*-PMMA composites.

The hot block is heated by two cartridge heaters inserted into the aluminium, and the cold block is cooled by water circulation. An enclosed channel on top of the aluminium blocks allows the sample to be drawn across the thermal junction. The sample holder is connected by a lead screw and a gearbox to a motor, which allows the sample to be drawn from the heated to the cooled section of the apparatus at a predetermined rate.

To form the polymer composites, solutions of *mNA* and PMMA of varying concentrations were prepared using toluene as a solvent. Thin films (30–40 μm) were cast on glass slides and allowed to dry. The film was then covered with a second glass slide and placed in the sample channel of the thermal-gradient device at the heated end. For the *mNA*/PMMA guest-host combination, the heated section was maintained at 150 $^{\circ}\text{C}$ (above the melting point of *mNA*); the cooled block was at 20 $^{\circ}\text{C}$. When the sample in the hot end had softened but not completely melted, it was drawn slowly across the thermal junction. When the appropriate conditions of temperature differential and drawing speed are optimized, the guest material

crystallizes in a line within the polymer film as it traverses the temperature gradient; the crystals appear as fibre-like needles aligned parallel to the drawing axis.

The optical quality of the composites was evaluated using optical microscopy, the intensity and angular distribution of helium-neon-laser scattering, and by determining the efficiency of second-harmonic generation (SHG). The experimental procedure for measuring scattering was routine. To determine SHG efficiencies, the sample was irradiated with 1,064-nm infrared radiation from a Nd:YAG laser; frequency-doubled (532-nm) visible light was detected and its intensity was measured by a photomultiplier.

We summarize here the results of a detailed and comprehensive study of the parameters that affect crystalline growth rates and alignment within the polymer host: dependence of these properties on concentration, temperature gradient, drawing speed and polymer host will be addressed elsewhere. The results described here were obtained when these factors had been optimized, and we include for comparison data from samples fabricated under less than ideal conditions.

Figure 1 shows a sample of *mNA* in PMMA under the polarizing microscope. The crystals of *mNA* adopt a needle-like habit, which is strongly aligned along the drawing axis (the direction of the thermal gradient). He-Ne laser scattering provides a more quantitative measure of optical quality; Fig. 2 shows the scattering intensity of 632-nm light as a function of angle for both an aligned and a partially aligned sample. For the aligned sample (70% *mNA* in PMMA), the angular scattering pattern is narrow and symmetric about 0 $^{\circ}$. The peak represents transmission at 632 nm of 88.7%. Higher peak transmissions are achieved using PVK as the polymer host, with a concomitantly narrower angular distribution (a result of improved refractive-index matching between guest and host). With improved growing techniques, we anticipate that losses could be reduced below 1 dB cm^{-1} for in-plane propagation.

The most revealing results come from study of the efficiency of SHG (Fig. 3) for the aligned and partially aligned samples. There is a striking increase in the SHG efficiency as the degree of crystalline alignment is improved. There is also a marked narrowing of the SHG angular distribution as crystal alignment increases, with the majority of the intensity being forward-scattered. The best-aligned sample gives an SHG intensity more than 400 times that of powdered potassium dihydrogenphosphate (KDP), a commonly used frequency-doubling material, at a thickness of 250 μm . Thus these composite materials represent a remarkably efficient frequency-doubling medium. A preliminary crystallographic study indicates that the crystals of *mNA* are about 2–3 times larger than the coherence length for SHG. Thus, improvements in SHG efficiency may be anticipated

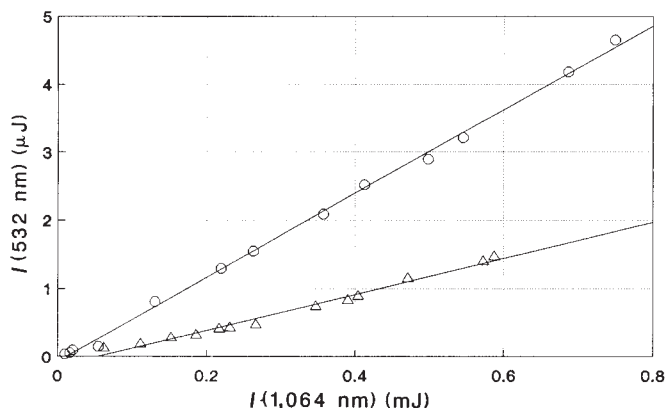


FIG. 3 The SHG efficiencies (incident wavelength 1,064 nm, emitted wavelength 532 nm) of partially aligned (Δ) and well aligned ($\circ$) *mNA*-PMMA composites.

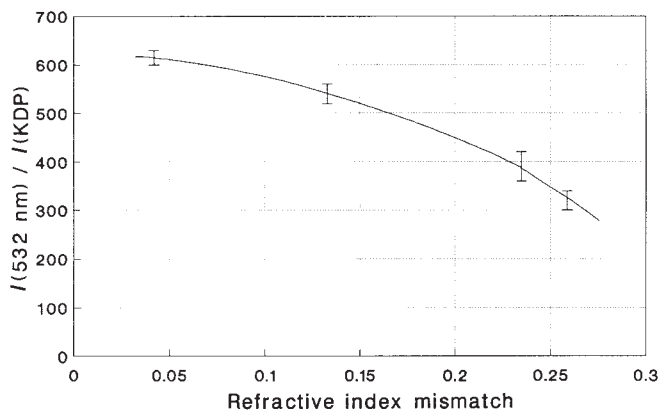


FIG. 4 Intensity of second-harmonic generation (incident wavelength 1,064 nm, emitted wavelength 532 nm) as a function of refractive-index mismatch for various *mNA*-polymer composites.

by using phase-matching techniques, such as birefringence or host index modification.

The SHG efficiency may be enhanced further⁸ by suitable choice of polymer host. A matching of the average value of crystalline refractive index with that of the host in a direction normal to the film plane may be achieved, and this results in improved optical homogeneity. Furthermore, SHG efficiency increases significantly as the refractive-index match is improved (Fig. 4). The origin of this improved efficiency is not yet fully established, but may be the result of reduced scatter for 532-nm radiation.

These composite materials have excellent chemical and optical stability. The samples used here have shown unchanged clarity, composition and SHG efficiencies for more than a year. This is considerably greater than the lifetimes of aligned composites produced by other methods and augurs well for their incorporation into optoelectronic devices⁹. Some damage does occur at high Nd:YAG power levels; this has not yet been investigated quantitatively, but there is a need for materials for use in low-power applications. □

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Diffuse volcanic emissions of carbon dioxide from Vulcano Island, Italy

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RECENT investigations on Mount Etna (Sicily)^{1–3} have revealed that volcanoes may release abundant carbon dioxide not only from their active craters, but also from their flanks, as diffuse soil emanations. Here we present analyses of soil gases and air in water wells on Vulcano Island which provide further evidence of such lateral degassing. Nearly pure carbon dioxide, enriched in helium and radon, escapes from the slopes of the Fossa active cone, adding a total output of 30 tonnes per day to the fumarolic crater discharge (~180 tonnes CO₂ per day). This emanation has similar He/CO₂ and ¹³C/¹²C ratios to those of the crater fumaroles (300–500 °C) and therefore a similar volcanic origin. Gases rich in carbon dioxide also escape at sea level along the isthmus between the Fossa and Vulcanello volcanic cones, but their depletion in both He and ¹³C suggests a distinct source. Diffuse volcanic gas emanations, once their genetic link with central fumarole degassing has been demonstrated, can be used for continuous volcano monitoring, at safe distances from active craters. Such monitoring

has been initiated at Vulcano, where soil and well emanations of nearly pure CO₂ themselves represent a threat to the local population.

Vulcano Island, in the Aeolian island arc, north of Sicily, is the emerged part of a 2,000-m-high active volcano built on the Tyrrhenian Sea floor. The Fossa active cone (391 m above sea level) was formed in early prehistoric times, in a 2.5-km-wide caldera (Fig. 1). Since its last eruption in 1888–90, it has remained the site of intense fumarolic activity⁴, mainly along the northern edge of the crater. Fumarolic degassing also occurs at and below sea level, along the isthmus connecting the Fossa cone to Vulcanello, a volcanic cone formed by submarine eruptions in 183 BC (ref. 5). Increasing activity has been observed over the past ten years, especially since 1985. The temperature of the Fossa crater fumaroles rose from 200 °C in 1978 (ref. 6) to nearly 500 °C in October 1988 (and >550 °C in July 1989) in connection with an increasing gas outflow, spreading of the fumarolic field and opening of new fractures⁷. Sea-level fumaroles maintain a maximum temperature of 100 °C. Their

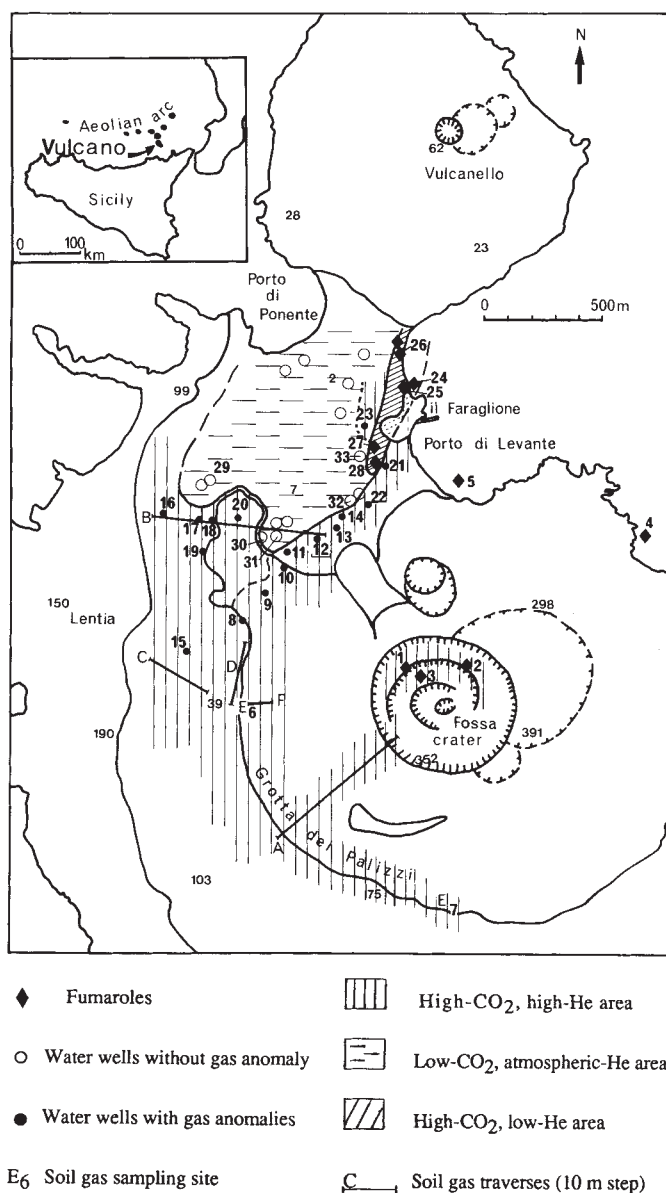


FIG. 1 Areal distribution of soil gas anomalies in the Fossa caldera of Vulcano in 1988 and location of sampled fumaroles, soil gases and well gases (Table 1).

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TABLE 1 Analysis of representative gas samples from fumaroles, soils and water wells on Vulcano Island

Site	Sample	T (°C)	CO ₂ (%)	He (p.p.m.)	He/CO ₂ (10 ⁻⁶)	²²² Rn (pCi l ⁻¹)	δ ¹³ C (‰)
1	Fumarole crater, rim fissure	235	75.8	7.5	9.9	1,710	-0.2
2	Fumarole F5, crate rim (09/88)	330	85.7	11.2	13.1	1,760	0.2
2	Fumarole F5, crater rim (10/88)	333	85.0	10.2	12.0		0.3
3	Fumarole 'blue', crater pit (09/88)	340	68.5	11.8	17.2	1,100	-0.5
3	Fumarole 'blue', crater pit (10/88)	370	91.0	6.7	7.4		-0.1
4	Submarine fumarole, Fossa cone (09/87)	86	98.0	16.3	16.6		-0.4
5	Submarine fumarole, Fossa cone (09/87)	58	98.0	6.7	6.8		-0.8
6	Soil gas, Grotta dei Palizzi		87.0	8.2	9.4	857	0.2
7	Soil gas, Grotta dei Palizzi		83.0	11.7	14.1	414	-0.3
8	Well gas, Palermo station		72.0	10.8	15.0	780	
9	Well gas, NW	82	79.6	10.6	13.3	1,588	
10	Well gas, Camping		48.8	8.5	17.4	415	
11	Well gas, Salvatore		67.2	10.2	15.2	1,065	
12	Well gas, Chantal	60	93.5	11.7	12.5	2,070	-0.7
13	Well gas, Chantal-N		85.9	10.1	11.8	1,200	
14	Well gas, tennis-S		54.8	7.7	14.1	515	
15	Well gas, Lentia 38		58.1	7.7	13.3	494	
16	Well gas, Lentia 22-1		26.9	6.8	25.3	265	
17	Well gas, Lentia 22-2		49.6	8.3	16.7	275	
18	Well gas, Lentia 22-3		54.1	8.6	15.9	194	
19	Well gas, Lentia 22-4		58.0	9.5	16.4	312	
20	Well gas, Reservoir		18.3	5.8	31.7	195	
21	Well gas, Porto Levante E	28	36.5	5.7	15.6	790	
22	Well gas, Strada		3.2	5.6	175.0	53	
23	Well gas, piazzetta Faraglione		40.0	7.6	19.0	765	
24	Submarine fumarole, Porto Levante	101	100.0	3.1	3.1	1,352	-3.2
25	Fumarole, Acqua Bollente (09/87)	100	98.0	2.9	3.0		-3.4
26	Beach fumarole, north pool	40	100.0	3.0	3.0	1,058	-3.4
27	Well gas, Vincenzino		82.5	4.6	5.6	1,012	-2.9
28	Well gas, Porto Levante SW		97.0	3.6	3.7	980	-2.7
29	Well gas, Villa Ponente		0.7	5.0	714.0	70	
30	Well gas, Communal 12-3		1.2				
31	Well gas, Maria 12-3		0.4				
32	Well gas, tennis-N		0.4				
33	Well gas, Levante		0.8				
	Atmosphere (Porto Ponente)	25	0.04	5.2	13,000	2	

The location of sampling sites is shown in Fig. 1. All but a few samples were collected between September and October 1988. Details of the sampling and analytical procedures are given in ref. 18. CO₂ was analysed with an infrared spectrometer (ADC, RFD-10,444), helium with both quadrupole (BALZERS-QMG200) and permanent magnet (ALCATEL-SM100) mass spectrometers, and radon with an α -particle counter (NARDEUX-SMIA70). The precision on their concentrations is $\pm 0.5\%$, 2% , and 10% , respectively. The carbon isotope ratio of CO₂ was measured with a double-collecting VG 602D mass spectrometer, after purification of the gas under vacuum. The results are given as $\delta^{13}\text{C}(\text{‰}) = [(R_s/R_{\text{std}}) - 1] \times 10^3$, where R is $^{13}\text{C}/^{12}\text{C}$ in the sample(s) and in the PDB standard (std). The accuracy is $\pm 0.1\%$.

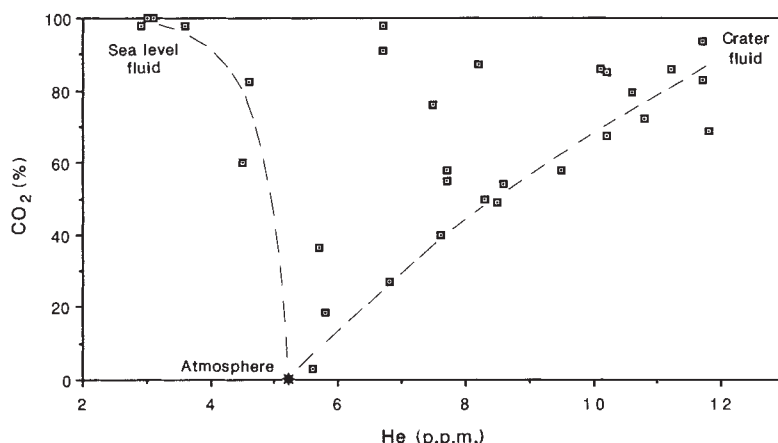
chemical composition is different from that of Fossa cone fumaroles (lack of SO₂, depletion in HCl, enrichment in H₂ and CH₄)^{4,6,8-11}, a feature generally attributed to low-temperature fractionation of the crater-type fluid in shallow aquifers⁸⁻¹¹. Isotope data¹²⁻¹⁴ show that both Fossa cone and sea-level fumaroles contain mantle helium, which may derive from a magma intrusion about 1 km wide and 2-3 km beneath the Fossa crater¹⁵. Degassing of this intrusion may thus affect a large area of the island at the surface.

Preliminary soil-gas investigations^{16,17} in 1984-87 revealed anomalous ground enrichments of CO₂ and/or He in the active part of Vulcano. Between September and November 1988, because of the increasing activity, we performed analyses of CO₂, He and ²²²Rn (together with N₂, O₂ and Ar) in soil gases, water-well atmospheres and fumaroles in this part of the island. Analysis was made either in-line or after sampling with both a vehicle-mounted quadrupole mass spectrometer³ and specific analysers (portable infrared spectrometer for CO₂, α -particle counter for radon). Soil gas fluxes were also measured in representative places, using a static (gas accumulation) method¹⁸. Carbon isotope ratios were determined on selected samples. The procedures and their accuracy are indicated together with the results in Table 1.

Extensive mapping of soil gases at 80 cm depth and analysis of water-well atmospheres demonstrate the occurrence of important gas leaks in several areas apart from the fumarolic zones.

Four distinct areas were identified: (1) Soil and well gases from the slopes of the Fossa cone are highly enriched in carbon dioxide, helium (up to 12 parts per 10⁶ by volume (p.p.m.v.)) and radon (up to 2,000 pCi l⁻¹). Their He/CO₂ and ¹³C/¹²C ratios are comparable to those of the crater fumaroles (Table 1), and therefore point to a similar volcanic origin. The CO₂ content of the volcanic ground ranges from several volume per cent up to 100% in some places, the highest values being found in a 40-m-wide belt, depleted in vegetation, that surrounds the base of the cone. The CO₂ flow rates, for CO₂ concentrations between 6.5% and 87% at 80 cm depth, range from 0.23 to 2.64 l m⁻² h⁻¹. Based on these data and their spatial distribution, we calculate a total output of 30 t CO₂ d⁻¹ ($\pm 20\%$) from soil degassing of the entire surface of the cone (excluding the fumarolic fields). This important outflow can be compared with that from the crater fumaroles. Airborne measurements in the crater plume in September 1984 (J. Carbonnelle *et al.*, unpublished data) yielded a flux of 43 t d⁻¹ for total sulphur which, for a mean C/S weight ratio of 4.0-4.5 in the fumaroles^{8,11}, gives a total crater discharge of 180 $\pm$ 10 t CO₂ d⁻¹. Although the present sulphur output may be higher than it was in 1984, our results thus indicate that diffuse emanation of carbon dioxide through the Fossa cone contributes significantly to the total CO₂ discharge; (2) Two traverses from the western foot of the Fossa to the caldera wall (B and C, Fig. 1) show a gradual decrease of the soil gas concentrations with increasing distance from the

FIG. 2 CO_2 plotted against He in fumarolic, soil and well gases of Vulcano Island. The data define a mixing field between three components: the atmosphere ($\text{CO}_2=0.03\%$, $\text{He}=5.2$ p.p.m.), the Fossa crater gas ($\text{CO}_2\geq 90\%$, $\text{He}\geq 12.5$ p.p.m.), and the sea-level gas ($\text{CO}_2\sim 100\%$, $\text{He}\leq 3$ p.p.m.).



cone— CO_2 decreasing to 0.3%, the local biogenic background—except for a strong, narrow anomaly in CO_2 , He and Ar (data not shown) which probably marks the deep bordering fault of the caldera. Soil gas profiling indeed provides a reliable means for detecting active, gas-draining fractures on volcanoes, as previously shown on Mt Etna^{3,19}; (3) A large area with little or no gas release extends between the Fossa and the bay of Porto di Ponente. Low $\delta^{13}\text{C}$ values of about -20% , suggestive of an organic source, were measured on soil CO_2 in this area¹⁷; (4) CO_2 -rich soil and well gases with high radon activity but lower-than-atmospheric helium content (≤ 3 p.p.m.) escape from an elongated zone trending 010° along the Fossa-Vulcanello isthmus (Fig. 1). Their He/CO_2 and $^{13}\text{C}/^{12}\text{C}$ ratios are both similar to those of nearby sea-level fumaroles and significantly lower than those of Fossa gases at the same time (Table 1). Such chemical and isotope contrasts, noticed separately in the fumaroles for the past ten years^{6,17,20}, may indicate the existence of another hydrothermal system, distinct from the Fossa one, as briefly mentioned below. The total CO_2 output by soil degassing in this area has not yet been evaluated but is likely to be lower than that from the Fossa cone.

It has been proposed⁸⁻¹¹ that the sea-level fumarolic gases have the same origin as the Fossa cone gases, but become depleted in their more soluble components (SO_2 , HCl) and thus enriched in the less soluble ones (H_2 , CH_4) while passing through shallow aquifers. If so, they should be richer, not poorer, in helium than the Fossa cone gases, because helium partitions strongly into the vapour phase during the boiling of water. Our results for these fumaroles and related soil and well gases conflict with this simple model. In the plot of CO_2 against He (Fig. 2), our samples define two distinct mixing trends between the atmosphere and either sea-level or Fossa cone fumarolic gas, some of them having an intermediate composition. Atmospheric dilution of the crater-type gas cannot produce the sea-level end-member. Dilution by entrained air dissolved in shallow aquifers (4.8×10^{-8} cm³ STP He per g H_2O in air-saturated water at 15°C , $^3\text{He}/^4\text{He}$ ratio = 1.4×10^{-6}) is a possibility, but cannot explain both the low helium content and high $^3\text{He}/^4\text{He}$ ratio^{13,14} of these gases (1.6×10^{-4} cm³ STP He per g H_2O and $5.6\text{--}7.0 \times 10^{-6}$, respectively, compared with 1.5×10^{-3} and $7.0\text{--}7.7 \times 10^{-6}$ in the Fossa cone fumaroles¹²⁻¹⁴). The isotope data demonstrate that helium in both sea-level and Fossa cone fumaroles is predominantly of mantle origin, contrary to the statement of Mazor *et al.*¹¹. Only two mechanisms could then account for the low abundance of this rare gas: (1) dilution by helium-free carbon dioxide from a non-magmatic source; and/or (2) late degassing of a helium-depleted magmatic system. A detailed discussion of these alternatives will be presented elsewhere (Allard *et al.*, manuscript in preparation). Here we will merely state that analysis of CO_2 and He in soil and well gases on volcanoes provides further information to that from fumaroles, allowing us to distin-

guish different hydrothermal systems and set limits on their areal extension.

At Vulcano, in a non-eruptive stage, diffuse emanations account for $\sim 15\%$ of the total CO_2 flux, a much smaller proportion than found on an erupting site such as Etna (P.A. *et al.*, manuscript in preparation). Compared with previous data^{16,17} our results indicate a rather similar general distribution but a much higher level of soil gas anomalies in 1988, which correlates with the recent increase in fumarolic activity at the crater. In many places the concentrations of CO_2 (and radon) in the ground, in water wells, or in both, are high enough to be a hazard. In the past decade CO_2 emanations at Vulcano have been responsible for occasional deaths of animals (rabbits, goats) and even of two children (Vincenzino well, site 27 in Fig. 1), and camping around the Fossa cone is now discouraged. We suggest that the hazard from such emanations has increased recently, especially in those inhabited areas at the foot of the cone, and should be carefully considered if the activity increases further. As illustrated by the 1979 catastrophe at Dieng, Indonesia²¹, a sudden release of large quantities of CO_2 , triggered by earthquakes, phreatic eruptions or flank collapse of the cone, would be difficult to anticipate. Continuous monitoring of CO_2 , He and Rn in a 'sensitive' water well at the base of the Fossa cone (site 12) was thus set up¹⁸. More generally, we emphasize that soil and/or well emanations of volcanic gas, once their genetic link with central fumarolic/magmatic degassing has been shown, provide appropriate conditions for continuous geochemical monitoring, at safe distances from active craters. □

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Sm–Nd evidence for the age and origin of a Mississippi Valley Type ore deposit

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MISSISSIPPI Valley Type (MVT) ore deposits represent the relatively common product of large-scale fluid transport in the continental lithosphere, yet the models for their genesis have been more controversial and unconstrained than those of any other class of giant ore deposit^{1,2}. Here we show that Sm–Nd isotope data can be used to determine the age and origin of an MVT deposit. Sm–Nd data for fluorites from the North Pennine orefield are difficult to explain unless some of the mineralization is of Mesozoic rather than the traditionally accepted Palaeozoic age. Furthermore, the Nd and Sr isotopic compositions of the fluorites do not support a variety of recent models that include derivation of the components from the mantle, the Lower Palaeozoic basement or the underlying buried granite which served to focus the flow of hydrothermal fluids.

The North Pennine orefield (or more strictly, that associated with the Alston block) is zoned above the buried 400-Myr-old Weardale granite^{3–6} and has a central fluorite zone (600 km²) that gives way abruptly to a barium-minerals zone^{7–10} (Fig. 1). The mineralization occurs chiefly in narrow fissure veins in flat-lying Lower Carboniferous limestones, shales and sandstones, but also extends into the Upper Carboniferous sediments and the underlying Lower Palaeozoic basement. Mineral assemblages comprise lead–zinc–iron sulphides, barite, fluorite, ankerite and quartz with minor chalcopryrite, primarily in veins but with many replacement features. The sulphides show a similar zone pattern with a localized central chalcopryrite–pyrite–pyrrhotite assemblage passing outwards into a galena–sphalerite assemblage^{11,12}. Maximum temperatures of ore deposition based on fluid-inclusion homogenization temperatures for early quartz indicate 190–230 °C, although most of the fluorite seems to have been deposited over a slightly lower temperature interval of 90–160 °C (refs 12–14). The compositions of the ore fluids approximate NaCl–CaCl₂ brines containing 19–23 wt% NaCl equivalents with Na/Ca ratios of 1.5–3.5 (ref. 13).

Data for twenty samples of fluorite from three veins (Slitt Vein, Ferneygill Vein and Groverake Vein) together with six samples of the underlying Weardale granite, Carboniferous sediments and Whin Sill obtained from the Rookhope borehole are presented in Table 1. Compared to other UK hydrothermal fluorites, the North Pennine fluorites are exceedingly rich in rare-earth elements (REEs) (up to 800 p.p.m. total REEs) and have high Tb/La ratios, typically 0.1–0.5 (ref. 13). Consequently, nearly all the fluorites have Sm/Nd ratios that are greater than (up to twice) chondritic, which allows for important constraints to be placed on the timing of mineralization. The Groverake and Ferneygill Vein fluorites have particularly high Sm/Nd ratios and are especially useful in this regard. Sm–Nd is therefore potentially superior to other isotopic methods such as U–Pb and Rb–Sr in that a major mineralization phase displays relatively large fractionations in parent/daughter ratio.

The ϵ_{Nd} values of the fluorite samples are displayed as a function of time in Fig. 2. The fields for Palaeozoic sediments of England and Wales¹⁵ and the northern England Lower

Palaeozoic granites, namely Weardale (Table 1), Shap, Eskdale and Cheviot¹⁶, are also shown. The latter define a reasonably uniform field with relatively high ϵ_{Nd} compared with the Palaeozoic sediments and fluorites. The Whin Sill (a quartz tholeiitic Permo-Carboniferous intrusion thought to be related to a failed rift of the European continent at 290 Myr (refs 17, 18)) has Nd isotopic compositions close to chondritic. This is far more radiogenic than that of the fluorites and immediately rules out the possibility of these tholeiites representing a source for the Nd in the mineralizing fluid.

The Nd isotopic compositions of the fluorites overlap those of the granites only at ages <100 Myr (Fig. 2). Hence, if the Nd were extracted in approximate isotopic equilibrium with its source rocks, the REEs can have been derived only from the underlying Weardale Granite, as suggested by Brown *et al.*¹⁹, if the mineralization is ≤ 100 Myr old. This seems to be somewhat unlikely because there is no significant mineralization in rocks of Jurassic or younger age anywhere in the United Kingdom. It is also difficult to reconcile with some older K–Ar and ⁴⁰Ar–³⁹Ar ages for hydrothermal clays of up to ~280 Myr (refs 20, 21). If the Nd in the fluorites was derived in isotopic equilibrium with Lower Palaeozoic sediments, the age of some of the mineralization has to be young, that is, <200 Myr in the case of the Groverake Vein, and <150 Myr in the case of the Ferneygill Vein. The data for the Upper Palaeozoic sediments impose similar constraints on the age of the fluorites except for the Carboniferous shale R110 (Table 1), which has unusually unradiogenic Nd. But even if such a shale contributed Nd to the fluorites, two of the Ferneygill fluorites are still no older than 220 Myr. The data for the Slitt Vein fluorites are far less conclusive in terms of age of the mineralization because of their lower Sm/Nd ratio.

Shepherd *et al.*¹³ presented a Rb–Sr isochron age of 206 ± 9 Myr for inclusion fluids in quartz from the Great Sulphur Vein. The initial ⁸⁷Sr/⁸⁶Sr of these inclusion fluids is virtually identical to the Sr isotopic compositions of the fluorites reported in Table 1. This, plus the similar fluid-inclusion chemistry, is most easily explained if the fluorite and quartz mineralizations are broadly co-genetic. We therefore consider that the most likely age for the fluorite mineralization is ~200 Myr, consistent with the Sm–Nd data presented above.

The Rb/Sr ratios of the North Pennine fluorites are so low (<0.01) that a genetic interpretation of the Sr isotopic compositions is insensitive to the exact age assumed for the mineralization. In Fig. 3, the Sr and Nd isotopic compositions of the North Pennine fluorites are shown, together with the fields of data for potential source reservoirs, assuming that the fluorites are 200 Myr old. The value of combining the Sr isotopic data

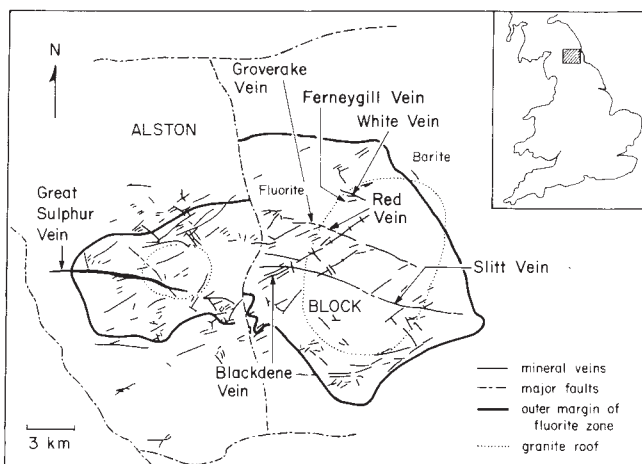


FIG. 1 Map of North Pennine orefield, showing locations of the veins that we sampled.

TABLE 1 Sm-Nd and Rb-Sr isotopic analyses of North Pennine fluorites and whole rock samples from the Rookhope borehole

Sample number	Locality	Lithology	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}\text{Rb}/^{86}\text{Sr}$ (atomic)	$^{87}\text{Sr}/^{86}\text{Sr}$ (atomic)	$^{87}\text{Sr}/^{86}\text{Sr}$ at 200 Myr	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd}$ $\pm 2\sigma$	$^{143}\text{Nd}/^{144}\text{Nd}$ at 200 Myr	ϵ_{Nd} at 200 Myr
North Pennine orefield													
B5	Slitt Vein	Fluorite	0.318	30.4	0.0303	0.71178 ± 6	0.71169	15.6	43.5	0.2172	0.51221 ± 2	0.51193	-8.9
B13	Slitt Vein	Fluorite	0.186	37.8	0.0142	0.71150 ± 10	0.71046	29.9	81.1	0.2232	0.51218 ± 2	0.51189	-9.6
B44	Slitt Vein	Fluorite	0.0729	53.1	0.00397	0.71138 ± 6	0.71136	38.6	92.8	0.2513	0.51223 ± 2	0.51190	-9.3
B53	Slitt Vein	Fluorite	0.121	28.8	0.0121	0.71094 ± 13	0.71090	6.25	13.3	0.2841	0.51228 ± 2	0.51191	-9.1
B75	Slitt Vein	Fluorite	0.165	41.6	0.0115	0.71139 ± 8	0.71136	3.72	12.0	0.1875	0.51220 ± 2	0.51196	-8.3
B11	Slitt Vein	Fluorite	0.101	35.4	0.00829	0.71091 ± 6	0.71089	9.49	17.9	0.3214	0.51232 ± 2	0.51190	-9.4
B48	Slitt Vein	Fluorite	0.244	44.9	0.0157	0.71166 ± 9	0.71161	25.2	71.0	0.2147	0.51218 ± 2	0.51190	-9.4
B74	Slitt Vein	Fluorite	0.238	60.3	0.0114	0.71360 ± 5	0.71356	7.64	30.0	0.1540	0.51202 ± 2	0.51182	-11.0
B3	Slitt Vein	Fluorite	3.85	30.0	0.372	0.71170 ± 4	0.71064	17.6	53.0	0.2009	0.51216 ± 2	0.51189	-9.5
B46	Slitt Vein	Fluorite	0.0877	41.1	0.00618	0.71149 ± 9	0.71147	26.9	70.9	0.2295	0.51219 ± 2	0.51189	-9.6
B50	Slitt Vein	Fluorite	0.0827	68.3	0.00350	0.71119 ± 6	0.71118	16.4	33.8	0.2936	0.51223 ± 2	0.51184	-10.5
929	Groverake Vein	Fluorite	0.144	41.5	0.0101	0.71092 ± 8	0.71089	14.6	21.9	0.4027	0.51229 ± 2	0.51176	-12.0
480	Groverake Vein	Fluorite	0.134	37.3	0.0104	0.71071 ± 12	0.71068	11.5	26.5	0.2611	0.51226 ± 2	0.51192	-9.0
853	Groverake Vein	Fluorite	0.122	65.9	0.00536	0.71069 ± 6	0.71068	15.1	33.4	0.2738	0.51227 ± 3	0.51191	-9.2
858	Groverake Vein	Fluorite	0.271	32.8	0.0240	0.71082 ± 17	0.71075	11.9	24.3	0.2972	0.51228 ± 3	0.51189	-9.6
933*	Ferneygill Vein	Fluorite						11.1	17.9	0.3763	0.51229 ± 1	0.51180	-11.3
943*	Ferneygill Vein	Fluorite						19.6	55.7	0.2125	0.51215 ± 1	0.51188	-9.9
949*	Ferneygill Vein	Fluorite						5.80	16.7	0.3494	0.51224 ± 1	0.51178	-11.7
951*	Ferneygill Vein	Fluorite						16.3	20.9	0.4712	0.51226 ± 1	0.51164	-14.4
1050	Ferneygill Vein	Fluorite	0.0851	40.7	0.00605	0.71142 ± 6	0.71142	15.8	22.0	0.4357	0.51221 ± 3	0.51164	-14.5
Rookhope borehole													
R105	2,554 ft	Granite	336	129	7.586	0.75273 ± 15	0.73115	3.55	17.4	0.1233	0.51214 ± 2	0.51198	-7.8
R107	1,330 ft	Granite	427	66.2	18.85	0.80331 ± 16	0.74969	3.23	16.5	0.1183	0.51209 ± 2	0.51193	-8.8
R108	1,200 ft	Limestone	1.42	424	0.00970	0.71073 ± 4	0.71070	0.395	1.45	0.1653	0.51207 ± 3	0.51185	-10.4
R110	1,000 ft	Shale	245	376	1.887	0.71823 ± 13	0.71286	8.85	49.4	0.1083	0.51173 ± 3	0.51159	-15.4
R111	847 ft	Dolerite	103	454	0.6570	0.70962 ± 8	0.70776	6.93	31.6	0.1326	0.51249 ± 2	0.51231	-1.3
R116	300 ft	Dolerite	22.1	444	0.1442	0.70655 ± 5	0.70614	6.65	28.4	0.1418	0.51260 ± 2	0.51241	+0.5

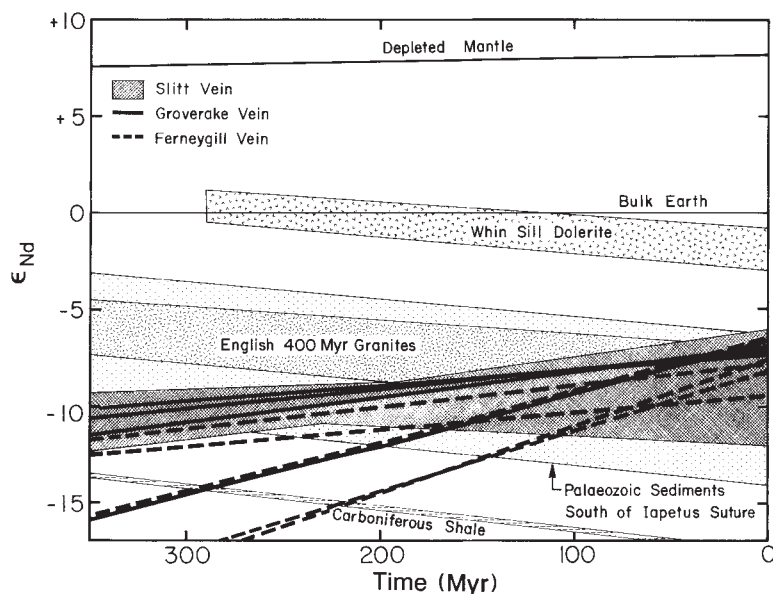
Rb, Sr, Sm and Nd were separated using standard ion-exchange procedures. All concentration measurements were performed using isotope dilution. Analyses marked with an asterisk in Table 1 were conducted at the University of Michigan using a VG Sector mass spectrometer as outlined elsewhere³⁹. All other analyses were performed at the Scottish Universities Research and Reactor Centre, East Kilbride, on a VG Isomass 54E mass spectrometer as described elsewhere⁴⁰.

with the Nd isotopic data is that it allows discrimination between potential source reservoirs that are not resolvable using Nd data alone. Although there is no very clear relationship between the Nd and Sr isotopic compositions of the North Pennine fluorites, the variations could reflect mixing of components from different reservoirs with different Sr/Nd ratios and isotopic compositions. The scatter observed does not support simple two-component fluid-mixing models, but rather suggests minor amounts of interaction between the fluid and a number of reservoirs. The North Pennine fluorites seem to have derived very little of their Nd or Sr (hence rare-earth and alkaline-earth budget) from the under-

lying Weardale granite, as has been advocated¹⁹. It is clear, however, that the Lower Carboniferous sediments are an ideal source reservoir for both Nd and Sr. The Sr isotopic compositions are important evidence in this respect because they distinguish the clastic-dominated black shale and turbidite Lower Palaeozoic successions from the carbonate-dominated platform and shelf Upper Palaeozoic successions with relatively unradiogenic Sr.

The chemical similarity between oilfield brines and the aqueous fluids trapped in fluid inclusions^{22,23} has led to basin-brine expulsion models²⁴⁻²⁸ for Mississippi Valley Type

FIG. 2 Plot of ϵ_{Nd} as a function of time for North Pennine fluorites and main crustal reservoirs in central Britain. Data from Table 1 and refs 15 and 16.



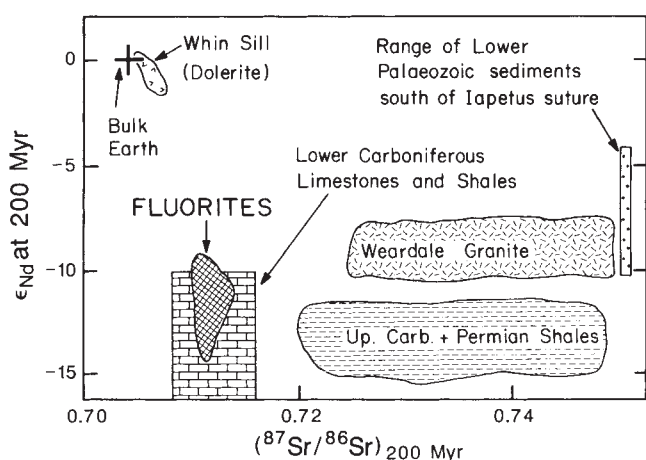


FIG. 3 Plot of ϵ_{Nd} against $^{87}\text{Sr}/^{86}\text{Sr}$ at 200 Myr for North Pennine fluorites and potential source reservoirs. Data from Table 1 and refs 13, 15 and 16.

deposits, which supersede the granite-related magmatic models of 20 years ago. The fluorite-rich Rossclaire- and Pennine-type deposits have proved distinctive and especially enigmatic², however, not least because of the problem of generating fluorine-rich basin brines at temperatures of up to 200 °C. For these, the older magmatic hypotheses have been remodelled to incorporate rifting or crypto-volcanic structures and the proximity of alkaline magmatism at depth to account for fluorine^{29,30}. For the North Pennine orefield, various basin-brine expulsion models, or refinements thereof, have been proposed^{7,13,19,31,32}, although the difficulty in identifying an unequivocal source for the fluorine has led to hybrid models that combine aspects of both basin-brine and rift-related hypotheses³³.

Although the exact age of mineralization has yet to be resolved, the range of K-Ar, ^{40}Ar - ^{39}Ar and Rb-Sr ages, endorsed by the Sm-Nd data, are consistent with fluid movements triggered by regional tectonic processes between the Carboniferous and the Jurassic. There is no regional evidence to support models of major lateral squeezing of fluids from the crust during the Mesozoic, which has been proposed as an explanation for formation of the MVT deposits of central and eastern United States in Upper Palaeozoic times³⁴⁻³⁶. Neither is there any evidence for coeval magmatic activity in the area. There is abundant evidence, however, for relatively sudden crustal-tension events and for widespread coeval hydrothermal activity^{37,38}. We consider it most likely, therefore, that the MVT deposits of the North Pennines were produced by anomalously deep penetration of the crust by fluids, concomitant with the production and reactivation of fracture permeability induced by unusual regional lithospheric tension. The Weardale Granite³, above which the mineralization is zoned⁴⁻⁶, clearly provided much of the geothermal energy for the hydrothermal activity. However, the data are difficult to reconcile with the hypothesis that the underlying granite dominates certain chemical characteristics (such as the REE budget) of the mineralization¹⁹. □

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Hydroperoxides in plants exposed to ozone mediate air pollution damage to alkene emitters

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OZONE is phytotoxic: it is damaging to cell integrity and photosynthesis^{1,2}, causing leaf necrosis³ and reducing crop yield⁴. It has been implicated in forest decline⁵, perhaps through interactions with stress ethene⁶. Here we show that organic hydroperoxides (ROOH), which are products of ozone-alkene reactions⁷⁻⁹, are present in the leaves of isoprene-emitting plants after exposure to ozone, but are not found in control plants grown in clean air. On the basis of earlier studies^{6,7,10}, we suggest that this reaction of ozone with biogenic alkenes to produce toxic ROOH could be one of the mechanisms by which damage to plants occurs. This could be particularly important in areas experiencing acidic deposition, where the stability of ROOH will be enhanced. This model may explain in part the die-back of tree species producing reactive alkenes, such as the red spruce, which emits isoprene¹¹⁻¹⁵ and monoterpenes¹⁶, and the Norway spruce and silver fir, which are both prolific monoterpene emitters¹⁷.

We have used HPLC with a hydroperoxide-specific detection system, which allows the separation and quantification of nine organic hydroperoxides and hydrogen peroxide in biological samples; the detection limit is 5×10^{-9} M as the ROOH^{18,19}. California poppies (*Eschscholzia californica*) were grown from seed in Teflon chambers housed in a controlled environment growth cabinet (at a temperature of 28 °C and a light regime of 12 h at intensity $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 12 h dark). Clean air

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TABLE 1 Isoprene emission potentials and concentrations of organic hydroperoxides in poppy extracts

	Hydroperoxides ($\mu\text{g per g dry weight}$)			
	H ₂ O ₂	HMHP	MHP	1-HEHP
Plants grown in clean air*				
	ND	ND	ND	ND
Plants grown in ambient air†				
	ND	ND	ND	ND
Plants exposed to 120 p.p.b. O ₃ ‡				
Experiment:				
a	ND	2.3	0.7	1.2
b	ND	2.0	0.3	0.1
c	ND	2.7	0.1	0.1
d	ND	2.1	ND	ND
e	ND	1.3	ND	ND
f	ND	1.1	ND	ND
Clean-air grown plants + 12 h O ₃ §				
	ND	1.7	ND	ND

* $n=10$; IE = $12.8 \mu\text{g per g (dry weight)} \text{ h}^{-1}$; dry weight = $2.038 \pm 0.487 \text{ mg}$.

† $n=3$; IE = $14.8 \mu\text{g per g (dry weight)} \text{ h}^{-1}$.

‡ $n=6$; IE = $2.9 \mu\text{g per g (dry weight)} \text{ h}^{-1}$; dry weight = $1.586 \pm 0.157 \text{ mg}$, $n=3$.

The analytical method was calibrated against H₂O₂; ND, not detected, as levels were below the detection limit of $0.02 \mu\text{g per g (dry weight)}$; dry weight is that of above-ground plants ($n=100$); experiments a–f are replicates; standard errors of the hydroperoxide concentrations, $\pm <5\%$; all samples comprised 10 plants; IE rates may be compared with typical emission rates from emitting tree species of $11.4 \mu\text{g per g (dry weight)} \text{ h}^{-1}$ for isoprene and of $3.1 \mu\text{g per (dry weight)} \text{ h}^{-1}$ for monoterpenes from coniferous trees at 28°C ³⁶.

(with concentrations of O₃, NO_x and hydrocarbons all below two parts per 10⁹ (p.p.b.)) was produced²¹, giving air exchange rates in the chambers of 2.5 min^{-1} . Ozone was produced by ultraviolet irradiation of pure O₂, added to the clean air supply as required and was measured photometrically.

Control plants were grown in clean air; others were fumigated with O₃ ($120 \pm 2 \text{ p.p.b.}$) for 10 h every day from germination. Plants were also grown in ambient air (mean [O₃] = 20 p.p.b.). After 15–18 days, leaves were collected and soaked in $10^{-3} \text{ M H}_2\text{SO}_4$ for 1 h at 20°C and the ROOH in the extracts was determined by HPLC. The potential emission rate of isoprene (IE) was determined by incubating the harvested leaves at 25°C for 12 h at a light intensity of $1,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ with headspace analysis by capillary gas chromatography with flame ionization detection.

Gross differences were observed between the control plants and those exposed to ozone: there was a distinct decrease in pigment, dry weights were 22% lower and IE was 77% lower in the ozone-exposed group. Hydrogen peroxide and the organic hydroperoxides were never detectable in leaf extracts from the controls. But ROOH was found in all plants exposed to 120 p.p.b. ozone for 10 h daily from germination: we identified hydroxymethyl hydroperoxide (HOCH₂OOH; HMHP), 1-hydroxyethyl hydroperoxide (CH₃CH(OH)OOH; 1-HEHP) and methyl hydroperoxide (CH₃OOH; MHP) at total concentrations up to $\sim 3 \mu\text{g g}^{-1}$, and at least two other unidentified hydroperoxides were present at lower concentrations. When plants were grown in clean air and then exposed to 120 p.p.b. ozone for 12 h, only HMHP was detected. These data are summarized in Table 1.

To establish whether these hydroperoxides are formed by reactions occurring inside or outside the leaf, we exposed velvet beans (*Mucuna sp.*) to ozone. Nearly all of the isoprene emitted by this species is from older leaves, young leaves having an emission rate 50–1,000 times lower. Although ROOH was not detected in young leaves (with an average IE of $0.06 \mu\text{g g}^{-1} \text{ h}^{-1}$),

TABLE 2 Isoprene emission potentials and concentrations of hydroperoxides in velvet bean extracts

O ₃ exposure period	Leaf position (node number)	IE ($\mu\text{g per g h}^{-1}$)	Hydroperoxides (ng per g dry weight)	
			H ₂ O ₂	HMHP
Control	4	0.017	ND	ND
	6	12.2	2	ND
1 day	4	0.010	ND	ND
	6	6.4	620	16
2 days	4	0.106	ND	ND
	6	6.1	168	ND
3 days	4	0.112	ND	ND
	6	5.1	90	4
4 days	4	0.042	ND	ND
	6	10.8	157	ND

IE, isoprene emission potential rate. ND, not detected, as levels were below the detection limit, $<1 \text{ ng per g (dry weight)}$ (compare with 20 ng per g in experiments with poppies, because there is much less baseline noise with bean extracts); O₃ concentration, 120 p.p.b. by volume, 10 h per day; all samples comprised 3 plants.

after exposure to ozone these compounds were always present in older exposed leaves on the same plant (average IE = $8.1 \mu\text{g g}^{-1} \text{ h}^{-1}$). The data obtained are shown in Table 2. We deduce that the hydroperoxides detected must be the result of reactions taking place between the alkene and ozone inside the leaf, not in the surrounding air, because this would lead to their deposition onto both emitting and non-emitting leaves.

The detrimental effects of hydrogen peroxide on plants are well known^{21–23}, but the toxic effects of organic hydroperoxides have not been studied in detail. But HMHP will inhibit some enzymes and it is mutagenic^{24–31}. If the rapid, irreversible inactivation of plant peroxidases by HMHP *in vitro*^{30,32} also occurs in intact plants, then this might enhance the toxic effects of the hydroperoxides. It is anticipated that the other hydroperoxides that we detect, being relatively unreactive with catalase³³, will also be phytotoxic. We suggest that the reactions of ozone with isoprene and other biogenic alkenes produces a suite of hydroperoxides in the plant tissue and that the free radicals observed in ozone-stressed plants³⁴ could be intermediates in their formation. This mechanism might contribute to air pollution damage to plants and trees and it is possible that such damage may be enhanced in acid environments, where the greater stability of the compounds³⁵ will facilitate their entry into tissue. This mechanism may therefore be of special importance in those areas currently experiencing forest die-back. □

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Rapid physiological adjustment of roots to localized soil enrichment

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SOIL microsites rich in available nutrients are an important source of mineral nutrients for plants in many environments^{1–5}. Patches in nutrient availability below ground is analogous to resource availability in canopy gaps above ground⁶. Although the physiological changes occurring in leaves exposed to sun and shade in canopy gaps are well known^{7–9}, we do not know any studies that show similar physiological changes in roots in enriched soil patches. Here we present evidence of large and rapid increases in the uptake kinetics of plant roots after creating nutrient-rich soil patches in the field. The mean rate of phosphate uptake at a given external phosphate concentration increased by as much as 80% for roots from enriched soil patches compared with roots of control patches treated with distilled water. The changes took place within days of patch treatment. This degree of plasticity was particularly notable for plants growing in soils of very low available phosphorus. These results showing rapid physiological plasticity of roots in fertile soil microsites have important implications for the theory and modelling of nutrient uptake in all soils.

We performed field experiments with three perennial species common to the Great Basin region of North America: a prominent shrub, *Artemisia tridentata* ssp. *vaseyana* (Rydb.) Beetle; an introduced tussock grass, *Agropyron desertorum* (Fisch. ex Lonk) Schult.; and a native tussock grass, *Agropyron spicatum* (Pursh) Scribn. and Smith (syn: *Pseudoroegneria spicata* (Pursh) A. Löve ssp. *spicata*¹⁰). All three species have vesicular-arbuscular mycorrhizae of the genus *Glomus*¹⁵.

The experiments were conducted in monoculture field plots of evenly spaced plants (0.5-m spacing) established 10 years earlier. Soils are Typic Haploxerolls¹¹, generally contain <6 p.p.m. bicarbonate-exchangeable phosphate¹², and have a solution phosphate concentration of ~1 µM. The average soil pH is ~8.0 (ref. 13), but the solution pH near roots is likely to be considerably more acidic owing to proton efflux from the roots¹⁴.

The first three experiments were conducted in May and June, 1989, with moist soil throughout the rooting zone. The fourth experiment was conducted one month later; the soil was drier and, therefore, water was applied before the experiment. Plants were actively growing during all experiments. We treated pairs of soil patches by placing, by the use of wicks, 750 ml distilled water on one side of a plant and 750 ml nutrient solution (45 mM NH₄NO₃, 20 mM KH₂PO₄) on the other side of the plant. NH₄NO₃ was used in addition to KH₂PO₄ because naturally occurring nutrient-rich patches presumably contain all three main plant nutrients (N, P and K). We removed samples of the

soil patches in soil cores (12 cm diameter, 25 cm deep) one week after treatment in the first three experiments and three days after treatment in the fourth experiment.

We sieved the excised roots from each soil patch out of the soil; retained those <0.5 mm in diameter (an approximate upper limit which we chose to confine the analysis to younger, finer roots), and separated them into five random subsamples. The excised-root assays were designed to provide relative comparisons of nutrient uptake; there is some evidence that the rate of uptake of phosphate in excised roots is more representative of intact-root uptake than is the rate of uptake of other ions, such as nitrate or potassium¹⁶. Both the time dependency and other factors limit the generality of this conclusion, however. We equilibrated subsampled roots in small cheesecloth bags in 0.5 mM CaCl₂ solution for 1 h at 20 °C¹⁷, and then placed them for 10 min in 1, 3, 5, 10 or 20 µM solutions of NaH₂PO₄ at 20 °C. The NaH₂PO₄ solutions contained 20–40 µCi l⁻¹ ³²P-labelled orthophosphoric acid. We used NaH₂PO₄ instead of

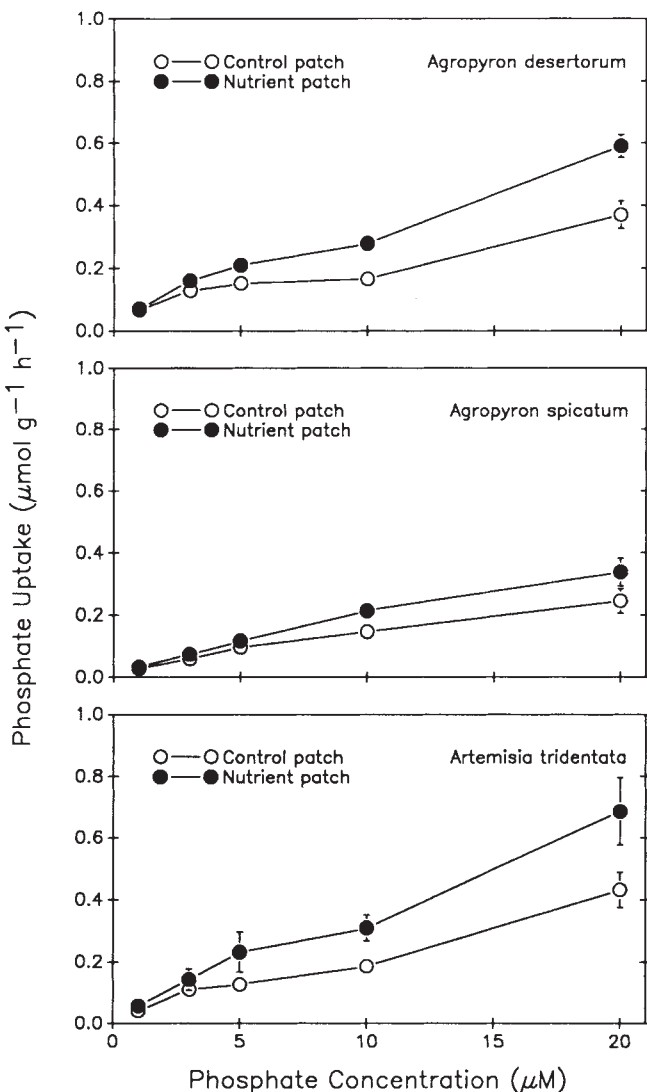


FIG. 1 The rate of phosphate uptake for roots from enriched and control soil patches as a function of the solution phosphate concentration (mean $\pm$ s.e.m.; $n=6$ for *Agropyron desertorum*, $n=8$ for *Agropyron spicatum* and *Artemisia tridentata*). Soil patches on opposite sides of plants in monoculture field plots were treated with 750 ml nutrient solution or distilled water. Samples of the soil patches were cored one week after treatment. Roots from each core were subsampled and immersed in radioactive phosphate solutions. The three single-species experiments were conducted at different times and in separate field plots; direct comparison of results among species is therefore inappropriate. The treatment effect was significant at $P < 0.05$ for each species (two-factor split-plot analyses of variance set out in blocks).

KH_2PO_4 to eliminate any effect of the important nutrient potassium on root nutrient status in different solution concentrations. (Although no data are available for the species used in our study, there are no significant differences between the uptakes of phosphate when either sodium- or potassium-phosphate solutions are used as phosphate sources for barley roots¹⁸.) We then rinsed the roots three times for at least 2 min in 200 μM unlabelled NaH_2PO_4 solution at 5 °C to replace any adsorbed radioisotope. Roots were dried in an oven, weighed, and counted by liquid scintillation using Cerenkov radiation¹⁹, with corrections for half-life and counting efficiency. All solutions were buffered to pH 6.0, were well mixed and aerated, and contained 0.5 mM CaCl_2 (ref. 20).

If plant roots respond physiologically to nutrient-rich soil patches, then increases in uptake capacity for soil nutrients are expected. Indeed, at every solution concentration, we saw striking increases in the mean rate of phosphate uptake per unit root mass—as much as 80% for roots from enriched versus distilled-water patches (Fig. 1). Each of the three species significantly increased its phosphate uptake capacity relative to that of its controls. The mean increases in the rate of phosphate uptake at the five external solution concentrations ranged from 7 to 58% for *Agropyron desertorum*, 16 to 47% for *Agropyron spicatum*, and 30 to 82% for *Artemisia tridentata*. Although the largest percentage increases generally occurred at 10 and 20 μM , increases in the mean rate of uptake at 3 and 5 μM were never less than 20% for any species.

Because we obtained such consistent differences in uptake kinetics one week after soil-patch treatment, we performed a follow-up experiment in which patches were cored three days after establishing enriched and control patches. A single plot

planted with *Agropyron spicatum* and *Agropyron desertorum* was used to compare directly the phosphate uptakes of the two tussock grasses. Again, the phosphate uptake rates of roots from enriched and control patches were clearly different (Fig. 2). The mean increases in the rates of phosphate uptake at the experimental solution concentrations ranged from 3 to 14% for *Agropyron desertorum*, and 16 to 46% for *Agropyron spicatum*.

Many factors are important for the uptake of soil nutrients by plant roots. Rooting density and root length are particularly important for the uptake of relatively immobile nutrients, such as phosphate, especially when soil phosphate levels are low and the buffering power of the soil is high^{21,22}. Plants growing in relatively nutrient-poor soils often have lower capacities for phosphate uptake than do plants growing in more nutrient-rich soils²³. Models and sensitivity analysis indicate that phosphate uptake could be more sensitive to changes in root surface area than to changes in kinetic parameters²⁴. Nevertheless, increased capacity for uptake of phosphate and other nutrients could contribute to rapid exploitation of the nutrients in the patches. In the relatively high solution concentrations within the patches, an increase in the Michaelis-Menten velocity parameter V_{max} is a likely explanation for the increased rate of phosphate uptake that we observed²⁵. This increase in V_{max} would probably be due to an increase in the number of phosphate carriers or pumps per unit weight of root^{18,25}.

Uptake capacity is greatly influenced by tissue nutrient concentrations and, as a result, plant demand for nutrients^{18,26}. Studies of uptake kinetics can be confounded by the inherent variability of plant nutrient demand among individual plants²⁷. This should not have confounded our study, because the same plants were exposed to both enriched and control patches.

Plant roots encountering fertile soil patches often branch and proliferate, thereby increasing local rooting density²⁸. The species that we studied differ in their relative ability to proliferate roots in nutrient-rich soil patches²⁹. *Agropyron desertorum* was shown to begin root proliferation within 1 day of patch enrichment, whereas *Agropyron spicatum* did not proliferate roots within 2 weeks of enrichment in any of the experiments²⁹. In the study described here, the excised roots of *Agropyron desertorum* could therefore have consisted of both newly proliferated and existing roots. But, at least for *Agropyron spicatum*, the increases in phosphate uptake capacity occurred in roots present in the patches before treatment.

Plants from relatively nutrient-rich soils are often thought to show more short-term morphological or physiological plasticity than do species in relatively nutrient-poor soils^{1,30}. Plants in poorer soils have, nevertheless, demonstrated dramatic growth responses to applied nutrients^{31–33}. Also, studies indicate that slower-growing species exploit short-duration unpredictable nutrient pulses more effectively than do faster-growing species^{30,34}. Although the three species we studied typically grow in soils in which phosphorus availability is low, they each had the physiological plasticity to rapidly and substantially increase their uptake capacity after phosphate levels in the soil increased. This striking plasticity implies that nutrient uptake capacity could be more important for mineral nutrient capture than is often thought. Explicit modelling of patchiness in soil nutrient availability might show that changes in uptake capacity are important for nutrient capture by plants in many soils. □

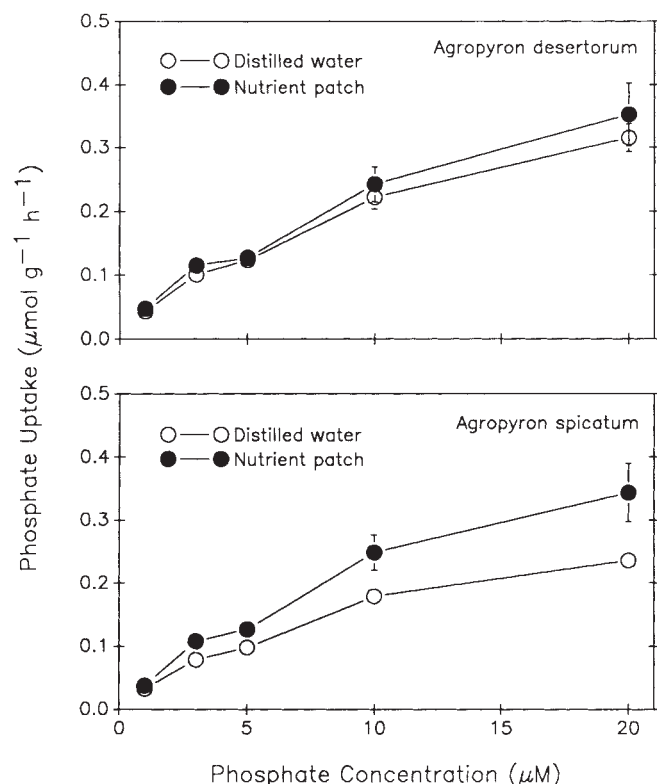


FIG. 2 The rate of phosphate uptake for roots from enriched and control soil patches as a function of the solution phosphate concentration (mean $\pm$ s.e.m.; $n=7$). The experimental procedure was the same as outlined for Fig. 1, except that soil patches were cored only 3 days after treatment, no *Artemisia* plants were tested, and results for the *Agropyron* species can be compared directly. The treatment effect was significant at $P<0.05$, but the species term was not significant (three-factor split-plot analysis of variance set out in blocks).

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Modulation of the motion aftereffect by selective attention

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THE motion aftereffect is a much studied and well documented phenomenon. After viewing a moving visual pattern for a period of time, the same pattern appears to drift in the opposite direction when it is stopped¹. Psychophysical experiments involving interocular transfer^{2,3}, dichoptic stimulation⁴, and motion aftereffects contingent upon other visual parameters such as colour^{5,6}, orientation⁵ and texture⁷, imply that the motion aftereffect is generated at the level of the visual cortex. It has been hypothesized that cortical neurons specialized for the detection of motion along a particular direction become 'fatigued' during the adaptation period so that the resting equilibrium subsequently shifts in the opposite direction to that of the adapting stimulus, giving rise to the sensation of the aftereffect^{8–13}. I have found that if observers are engaged in a separate discrimination task superimposed on a moving textured background, the subsequent motion aftereffect to the background is considerably reduced. It seems that motion aftereffects are susceptible to attentional mechanisms.

The attentional task in my experiments involved alphanumeric discrimination; the observer was required to strike a key every time a numeral appeared within a small window, on which the observer fixated, in the centre of the display (Fig. 1). The appearance of any alphabetical characters required no response. The characters and numbers were chosen randomly and presented at an alternation rate of 4 Hz without any intervening blank intervals. The probability of a numeral appearing was 1/8. During the discrimination period, the textured background moved in one of the four cardinal directions at 5 deg s⁻¹. Several different speeds (range: 2–7.5 deg s⁻¹) were used in control experiments with all of them showing similar results. Five naive observers viewed the display from a distance of 57 cm for 60 s, after which the entire stimulus became stationary and the alphanumeric alternation ceased. The observers were then required to report the moment the motion aftereffect ceased by striking a key, which prompted a computer-controlled stopwatch to report the duration of the aftereffect. A total of 5 trials for each of the experimental conditions described below was randomly pre-

sented. Only trials in which observers reported >95% of the numerals that appeared were accepted, thereby ensuring a high level of attentiveness to the discriminandum.

Three aspects of attentional modulation were examined. The first case documents the attenuation effect by comparing the duration of the motion aftereffect following alphanumeric discrimination with that of the control. The control experiment was passive viewing of the alphanumeric window; characters and numbers were alternated as before, but the observer was not required to take any action and merely to treat it as a fixation point. All five observers reported a significantly weaker motion aftereffect following the attentional task and showed reduced aftereffect durations compared with the control (Fig. 2a). A two-tailed *t*-test showed the differences between the control and task aftereffect durations to be highly significant ($P < 0.001$) for all subjects except number 5, for whom it was statistically significant ($P < 0.005$). Eye movement records (Fig. 3) did not show any significant differences between the two conditions except for a slight decrease in the background-induced optokinetic nystagmus frequency and amplitude during the attentional task. In a series of control experiments, I maintained the alphanumeric alternation throughout the test period for both of the conditions described. I observed no significant differences in the aftereffect durations from these results, indicating that the attentional modulation is not merely an effect of an altered attentional state between the adapting and test periods.

In the next case, the experiment was slightly modified to examine the effect of spatially dislocating the locus of attention from the fixation point. It is well known that the two need not be spatially coincident and that attention can be shifted across visual space in the absence of any accompanying foveating eye movements^{14–16}. In the context of the present experiment, the disassociation of fixation and attention allows their individual effects on motion adaptation to be expressed. The display in the preceding experiment was used with the addition of a small red fixation point, which is placed 2 deg arc to the right of the alphanumeric window. Motion aftereffect duration following an alphanumeric discrimination (attentional task) was compared with the control condition, which required only passive fixation. In both conditions, observers fixated the small red spot. All other aspects of the experiment remained unchanged. The same five observers participated in this experiment; four of them

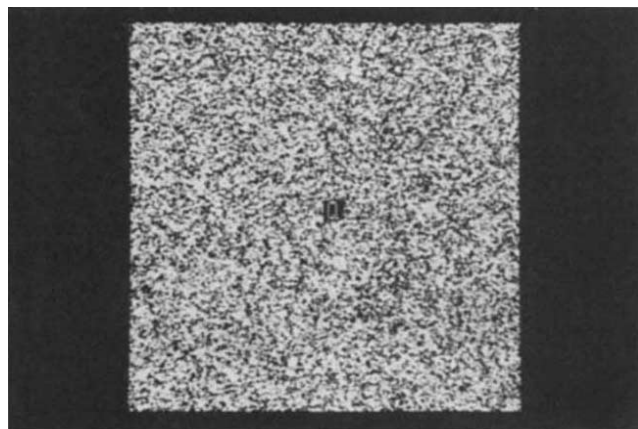


FIG. 1 Motion adaptation display with attentional window. Observers fixated the central window either passively or performed an alphanumeric discrimination task. During both conditions, the textured background moved in one of four directions at 5 deg s⁻¹.

METHODS. A PC installed with a graphics board (Number Nine Computer Corp.) generated the displays on a Zenith colour monitor. The adapting and alphanumeric windows were squares with sides subtending 11 deg arc and 0.5 deg arc respectively. The moving background had an average luminance of 130 cd m⁻².

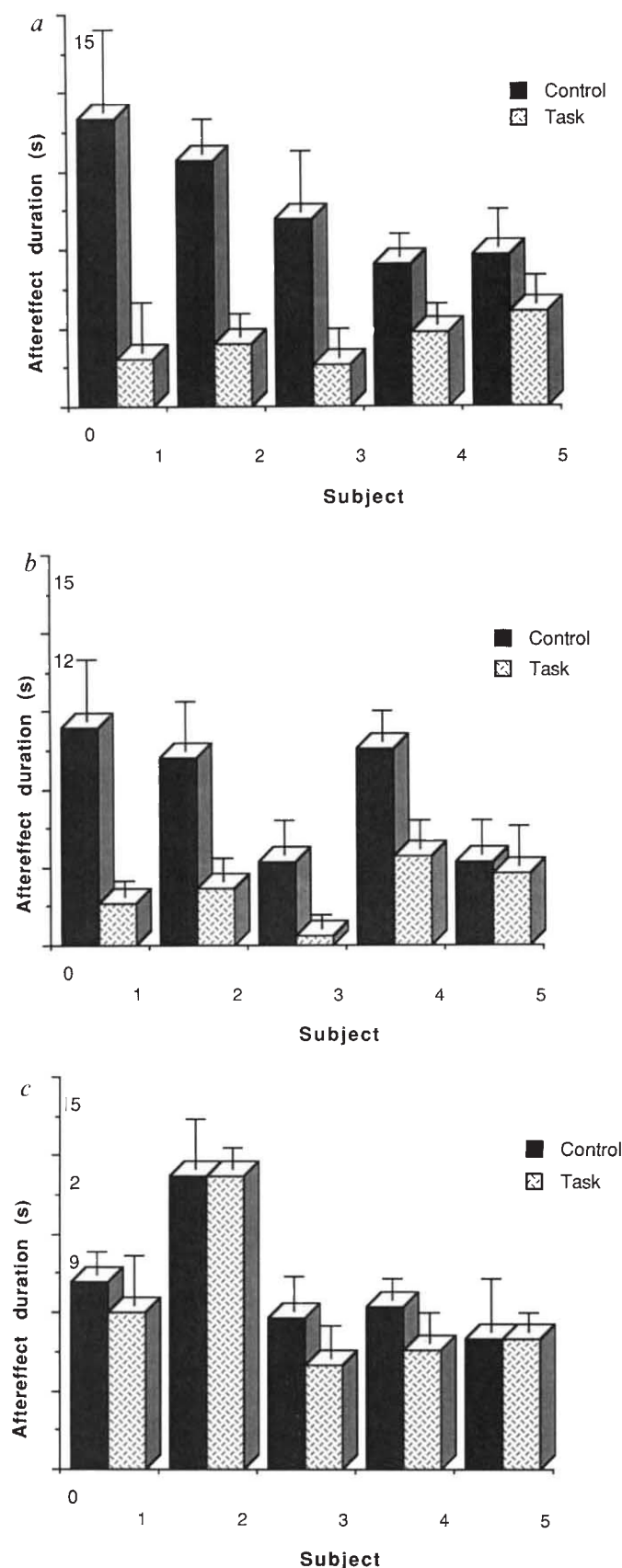


FIG. 2 Mean aftereffect durations following attentional and control tasks (5 trials per condition). Error bars indicate one s.d. *a*, Observer fixated a small central window where the discriminanda appeared. Fixational and attentional loci were coincidental. *b*, Attentional window offset by 2 deg to the left of the fixation point. *c*, Colour discrimination of the background. See text for details of the experiments.

reported a significantly reduced motion aftereffect (Fig. 2*b*) with the attentional task as compared with the control. For these observers, the difference in the data for the two conditions was highly significant ($P < 0.001$).

In the final case, parameter-specific attention was examined. The observer was not required to restrict attention on a localized region of space, but rather to the colour of the moving background. The white component of the textured background was made to be either red, orange, yellow or green and was randomly altered between these at 4 Hz. It was found that the false-alarm rate significantly increased in this and the previous experiments if faster alternation rates were used. By having the subjects perform at alternation rates just above threshold in all three experiments, differences in the difficulty of the discrimination task were considered to be minimized. All four coloured patterns were made to be equiluminant by the technique of heterochromatic flicker photometry on the individual observers. The observer's task was to strike a key every time a red background appeared while fixating on a spot in the centre of the display. The probability of the red background appearing was 1/4. The

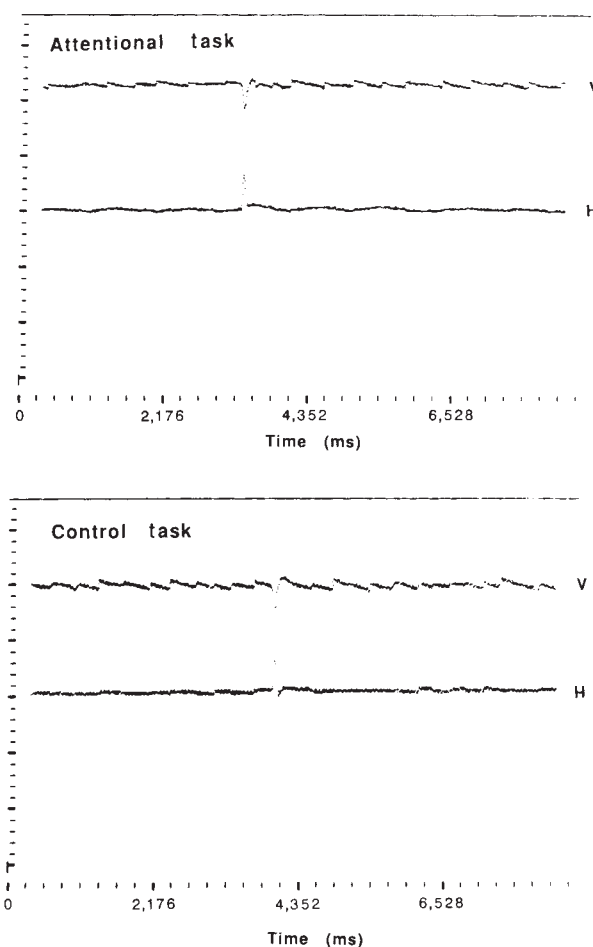


FIG. 3 Eye movement records during an alphanumeric discrimination task (upper) and the control (lower). In each record, the upper trace shows vertical and the lower trace shows horizontal eye movements. The adapting pattern moved vertically downwards. The deflections in the middle of the records were caused by an eye blink. Each small tick on the y axis represents 30 min arc.

METHODS. A variation of the magnetic search coil technique was used to obtain eye movement records^{22,23}. The observer was placed within a magnetic field which induced current upon eye movements in a search coil that was implanted within a contact lens (Skalar, Holland). The contact lens was placed in the left eye which was anaesthetized with Proparacaine HCl (0.5%) for the duration of the experiment. Several trials of the attentional and control tasks were performed during a period of 30 min.

control condition was passive viewing of the changing background colours without making any responses. All other aspects of the experiment remained unchanged. As Fig. 2c shows, after-effect durations were largely unaffected by performing the colour discrimination task during the adaptation period. A two-tailed *t*-test failed to find a significant difference in the data for the two conditions for all five observers. Colour discrimination of the background during the test period did not significantly alter the results. It seems that forced attention on an aspect of the stimulus that is not directly concerned with movement does not influence the aftereffect in any significant way, provided that it is in the same part of the visual field as the moving stimulus.

If motion aftereffects are a consequence of depressed activity in a population of directionally selective neurons during the post-adaptive period¹¹⁻¹³, then the results reported here would imply that the adapting pattern has a reduced effect on the same neurons if the visual system actively attends an alternative stimulus. The determinant of this in turn could be that non-attended visual stimuli are less effective in driving those neurons in the first place, so that the subsequent neural events that produce the aftereffect sensation are not initiated. Evidence for attention gated cortical processing has been shown in a number of reports and is thought to represent a mechanism of filtering out irrelevant information¹⁷⁻¹⁹. There are, however, no reports as yet of attentional effects on firing rates in motion-sensitive neurons or on the neural adaptation process.

The results reported here have certain implications on the site of the motion aftereffect. Although it is generally accepted that motion aftereffects have a cortical locus²⁻⁴, it now seems that

this is one in which cortical attentional mechanisms have considerable influence. Although tentative, the physiological evidence to date has revealed attentional modulation of neuronal activity only at sites beyond striate cortex¹⁷⁻¹⁹ (area 17, V1). Furthermore, recent psychophysical evidence for an extrastriate contribution to the motion aftereffects^{20,21} is consistent with the results presented here. □

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Interaction between neuropeptide Y and noradrenaline on central catecholamine neurons

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DESPITE their widespread occurrence in the central nervous system, interactions between co-localized transmitters and their receptors remain poorly understood. Noradrenergic neurons of the nucleus locus coeruleus contain the peptide co-transmitter neuropeptide Y (refs 1, 2). In locus coeruleus cells, stimulation of α_2 -adrenoceptors^{3,4} or opioid μ -receptors^{5,6} increases a potassium conductance and thereby leads to hyperpolarization and inhibition of spontaneous firing. Coupling between these receptors and the inward rectifying K⁺ channels involves a pertussis toxin-sensitive GTP-binding protein (G_i or G_o)⁷. Here we investigate whether the neuropeptide Y and α_2 -receptors of locus coeruleus neurons interact with one another. When administered alone, neuropeptide Y reduces the discharge of action potentials, probably by increasing the permeability of the membrane to potassium ions through the activation of a G protein; this effect is reduced in the presence of α_2 -adrenoceptor antagonists. Moreover, the peptide selectively increases the hyperpolarizing effect of α_2 -agonists, but does not enhance responses to opioid μ -agonists. We suggest that noradrenaline and its co-transmitter neuropeptide Y stimulate separate receptors, which influence each other in a specific way.

Slices containing the caudal locus coeruleus (LC) were prepared from the rat pons; recordings were from 56 cells. In two slices, neuropeptide Y (NPY) at 0.1 and 1 μ M was tested on the same neuron: the lower concentration depressed the discharge of action potentials, whereas the higher produced a slight hyperpolarization of 2.2 and 2.8 mV, respectively. As substantial quantities of the peptide are needed for bath-application, we subsequently used 0.1 μ M NPY, which inhibits the firing rate

by $40.6 \pm 5.9\%$ ($n = 56$; $P < 0.1$, Student's *t*-test; Fig. 1). The input resistance of LC neurons was 166.4 ± 23.4 M Ω ; this value decreases by $9.9 \pm 2.6\%$ in the presence of 0.1 μ M NPY ($n = 6$; $P < 0.05$; see legend to Fig. 1).

When applied twice with an interval of 5-14 min, 0.1 μ M NPY causes a reproducible depression of the spontaneous firing ($n = 7$). The α_2 -antagonist rauwolscine⁸ (1 μ M) by itself has no statistically significant effect ($-32.5 \pm 26.1\%$; $n = 6$; $P > 0.05$). Thus, a tonic control of neuronal activity by endogenously released noradrenaline does not seem to operate under these *in vitro* conditions. But at 1 μ M, rauwolscine decreases the effect of 0.1 μ M NPY ($54.3 \pm 13.3\%$ before, and $22.5 \pm 5.0\%$ after, the application of NPY; $n = 6$; $P < 0.05$; Fig. 1).

In the following experiments, we used concentrations of α_2 (ref. 4) and opioid⁶ agonists that produce about one third of the maximum hyperpolarizing response to these compounds. The effect of noradrenaline at 30 μ M is larger and longer-lasting in the presence of 0.1 μ M NPY (9.6 ± 0.9 mV) than in its absence (7.1 ± 0.8 mV; $n = 12$ for each; $P < 0.01$; Fig. 2). Moreover, this enhancement is completely reversible on washing out NPY. Also, the response to the selective α_2 -agonist UK14304 (ref. 9) at 0.1 μ M is increased by 0.1 μ M NPY (9.1 ± 1.5 mV, before, and 13.1 ± 1.7 mV after, drug application; $n = 7$ each; $P < 0.01$). Unexpectedly, the effects of opioid agonists are unchanged by 0.1 μ M NPY ((D-Ala₂, D-Leu₅) enkephalin, 0.03 μ M; 6.7 ± 0.8 mV without, and 6.5 ± 1.3 mV with, 0.1 μ M NPY; $n = 5$ each; $P > 0.05$), or even decreased ((Met₅) enkephalin, 0.3 μ M; 10.0 ± 1.3 mV without, and 6.8 ± 1.8 mV with, 0.1 μ M NPY; $n = 8$ for each; $P < 0.01$).

The intracellular injection of non-hydrolysable analogues of either GTP or GDP interferes with the coupling of receptors to G proteins¹⁰. GTP- γ -S causes the normal reversible responses to agonists to become irreversible, whereas GDP- β -S blocks the agonist effects. We found that in LC neurons impaled with microelectrodes containing 2 mM GTP- γ -S, the spontaneous discharge of action potentials stops and a slow hyperpolarization develops, even in the absence of noradrenaline ($n = 3$). It was therefore impossible to investigate any alteration in the firing rate by NPY, although noradrenaline becomes gradually less

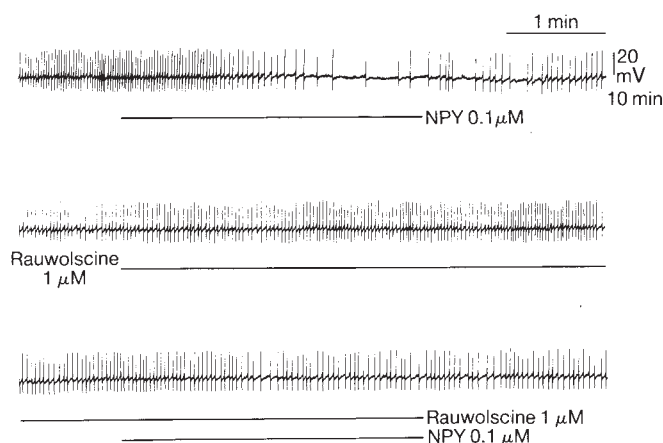


FIG. 1 Rauwolscine-sensitive depression by NPY of the spontaneous firing of LC neurons. NPY at $0.1 \mu\text{M}$ was in contact with the slices for 3 min. Its effect is expressed as per cent inhibition by comparing the action potential frequency during the third minute after drug application with the frequency during the last 2 min before drug application. Rauwolscine at $1 \mu\text{M}$ antagonizes the effect of $0.1 \mu\text{M}$ NPY. Drugs were present in the perfusing medium over the periods indicated by the horizontal bars.

METHODS. Slices from the rat pons (thickness, $250\text{--}400 \mu\text{m}$) containing the caudal part of the LC, were prepared as described¹⁴. Slices were submerged in a continuously flowing (2 ml min^{-1}) medium of composition (in mM): NaCl, 126; KCl, 5; NaH_2PO_4 , 1.2; MgCl_2 , 1.3; CaCl_2 , 2.4; NaHCO_3 , 25 and glucose, 11. The medium was saturated with 95% O_2 and 5% CO_2 and maintained at $35\text{--}36^\circ\text{C}$. LC neurons were impaled with glass microelectrodes filled with 2 M KCl and with tip resistances of $50\text{--}100 \text{ M}\Omega$. Recordings were amplified and displayed by conventional methods. The resting membrane potential of 56 neurons was $-53.0 \pm 0.4 \text{ mV}$. All cells fired spontaneously with a frequency of $0.79 \pm 0.10 \text{ Hz}$. In some experiments the membrane potential of LC neurons was raised by $7\text{--}12 \text{ mV}$ from rest by injecting a constant hyperpolarizing current, thereby preventing the discharge of action potentials. In addition, hyperpolarizing current pulses of constant amplitude and 300-ms duration were delivered at a frequency of 0.1 Hz to determine the apparent input resistance.

effective on repeated application and eventually, after 18–23 min, completely loses its activity. But when neurons are impaled with an electrode containing 2 mM GDP- β -S, the firing does not stop ($n = 3$; Fig. 3). Under these conditions, the effect of noradrenaline also decreases on repeated application and, at the same time, NPY becomes inactive.

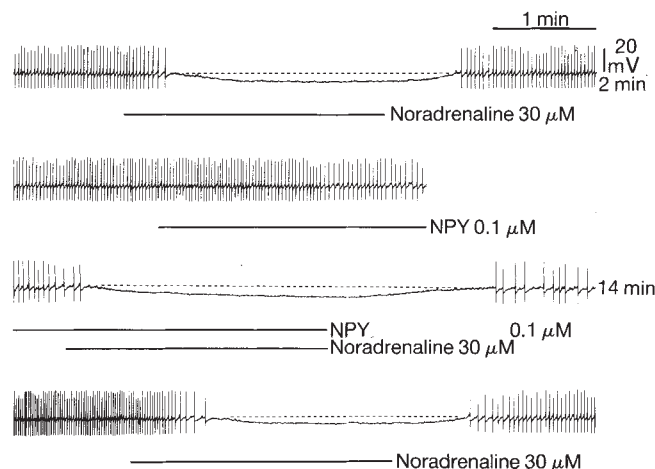


FIG. 2 Potentiation by NPY of the noradrenaline-induced hyperpolarization. The concentration of noradrenaline ($30 \mu\text{M}$) was chosen to produce about one third of the maximum response to α_2 -agonists⁶. Note that $0.1 \mu\text{M}$ NPY decreases the neuronal firing only slightly, but enhances both the amplitude and duration of the hyperpolarization caused by noradrenaline. The effect of NPY is completely reversible on washing it out. Drugs were in perfusing medium over the periods indicated by the horizontal bars.

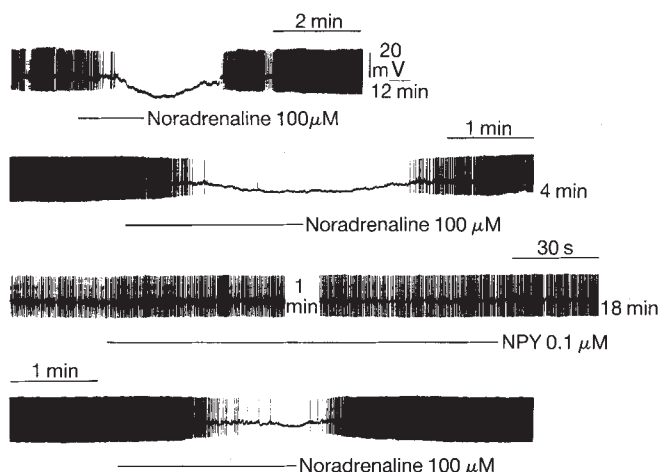


FIG. 3 Inhibition of the hyperpolarization caused by noradrenaline and of the decrease in frequency produced by NPY in the presence of intracellular GDP- β -S. A microelectrode containing 2 mM GDP- β -S and having a tip resistance of $57 \text{ M}\Omega$ was used for recording. The upper trace starts 1 min after the successful impalement. Noradrenaline at $100 \mu\text{M}$ and NPY at $0.1 \mu\text{M}$ were in the perfusing medium for the periods indicated by the horizontal bars (note the different timescales in the traces). In this type of experiment, the resistance of the electrode has to be low ($<60 \text{ M}\Omega$) to facilitate diffusion of GDP- β -S into the cell.

We have described the effects of NPY on the electrophysiological properties of central catecholamine neurons using LC cells, which store the peptide and noradrenaline together^{1,2}. NPY at $0.1 \mu\text{M}$ reduces spontaneous firing and produces a small but significant reduction in input resistance. The ions involved might be potassium and not chloride as the microelectrodes were filled with KCl, and under these conditions an increased permeability of the membrane to Cl^- depolarizes, rather than hyperpolarizes, LC neurons¹¹. When GDP- β -S-containing electrodes are used for recording, K^+ channels may be opened as a result of activation of a G protein of the type that operates with α_2 -adrenoceptors and opioid μ -receptors⁷. This idea is favoured by the finding that, in rats, the hypotension induced by intracisternal injection of NPY and the α_2 -agonist clonidine is reduced by pretreatment of the animals with pertussis toxin¹². The cardiovascular effects of both agonists were attributed, at least partly, to the inhibition of catecholamine neurons in the medulla oblongata¹³.

Two observations indicate that there could be a selective interaction between the NPY and α_2 -receptors. First, rauwolscine antagonizes the NPY-induced depression of firing. Second, NPY potentiates the hyperpolarizing effect of noradrenaline and UK14304 at α_2 -adrenoceptors⁴, but not of (Met₅)enkephalin and (D-Ala₂, D-Leu₅)enkephalin at opioid μ -receptors⁶. The site of interaction between NPY and α_2 -receptors might be in the neuronal membrane, between the receptors themselves, or in the common signal-transduction system. □

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Dystrophin gene transcribed from different promoters in neuronal and glial cells

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It has been shown that the dystrophin gene, which is defective in patients with Duchenne and Becker muscular dystrophy (reviewed in ref. 1), is transcribed in brain from a specific promoter that is different from the one used in muscle^{2,3}, and so the two types of transcripts differ at least in their first exon. We recently found that the dystrophin gene is expressed at a higher level in primary cultures of neuronal cells than in astro-glial cells derived from adult mouse brain⁴. Here we investigate the use of two different promoters in each cell type. Our results demonstrate that the brain-type promoter of the dystrophin gene is highly specific to neurons, in which there is a significant increase in the amount of brain-specific messenger RNA during the course of *in vitro* maturation. By contrast, the muscle-type promoter is active in a wider

range of cell types, including not only striated and smooth muscle, but also glial cells to a lesser extent, and probably neurons.

The exquisitely sensitive polymerase chain reaction (PCR) has proved very useful for the investigation of the low-abundance dystrophin mRNA⁵. By selecting appropriate primers in different exons it is possible to explore any segment of the very long (14 kilobases) mature transcript. Moreover, this method can be quantitative, provided that the following conditions are respected: (1) the PCR-amplified products must be estimated in the exponential phase of the amplification process; (2) the results must be standardized by comparison with an added exogenous mRNA that is reverse-transcribed and co-amplified in the same tube as the sample under analysis⁴.

We used the PCR to quantitate the relative amount of dystrophin mRNAs specifically transcribed from muscle and brain promoters in total RNA extracted from cultures of mouse brain neuronal and astro-glial cells^{6,7}. The primers were a common, forward primer, located in exon 2, and two specific reverse primers, located in the first muscle and brain exon, respectively (Fig. 1a). Sizes of the amplified fragments were in the same range so that yields were similar, a mandatory condition for comparative quantitation of the two mRNA species⁴. Figure 1b shows the results after 15 cycles of PCR amplification of either the brain-type fragment (81 base pairs (bp)) or the muscle-type fragment (93 bp), together with a fragment encoding L-pyruvate kinase (L-PK) (67 bp), used here as the internal standard. In assays with total RNA from brain or from cultured neurons (7 days old), we found that both promoters were functional, but

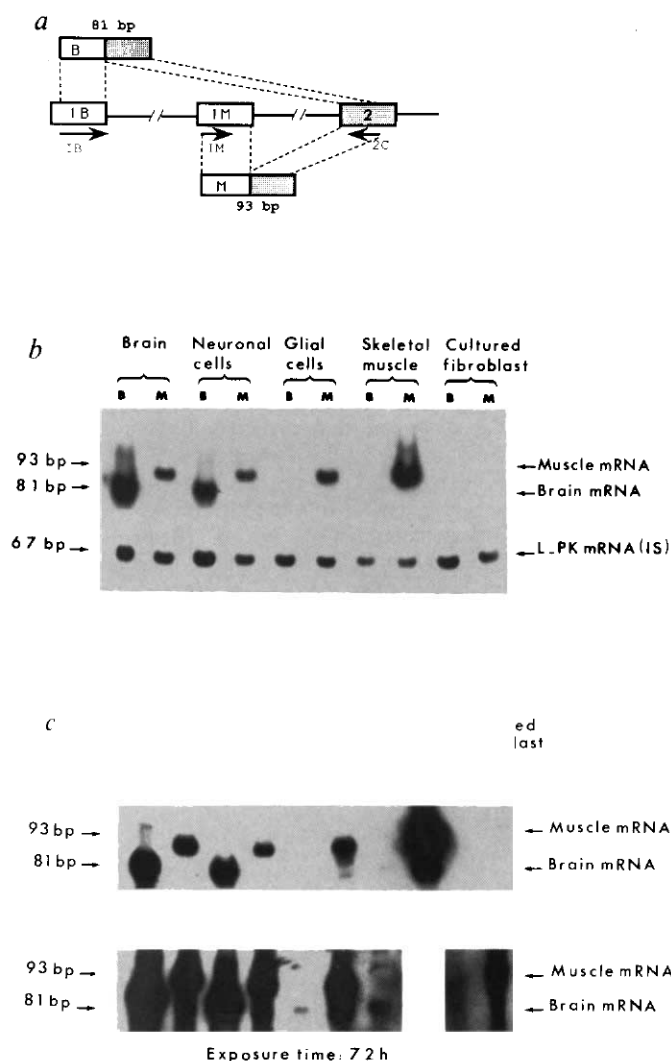


FIG. 1 Promotion of the dystrophin gene in muscle and cultured neuronal and glial cells. a, Position of the different oligonucleotide primers of mouse dystrophin exons and size of amplified fragments. The brain-type transcript (B) was amplified using primers 1B and 2C. 1B, Brain-specific 22-mer (5'-AAAACAGCTGGCATGGAAGATG-3') designed from the published sequence of the brain-specific first exons from man and rat (nucleotides 1-19) (refs 2, 3) and of the mouse second exon (nucleotides 20-22). As the sequence of the mouse brain-specific first exon is not yet known, we designed a primer in which the three 3' nucleotides were actually of mouse origin to ensure an efficient extension by *Taq* polymerase. 2C, Primer complementary to a sequence of the second exon (5'-CTTAGAAAATTGTGCA-TTTATCCA-3'). The muscle-type transcript (M) was amplified using primer 1M, which is identical in sequence to the muscle first exon 1M (5'-ATGCTTTGGTGGGAAGAAGTAGAG-3'), and primer 2C, which is complementary to a sequence of the second exon. b, Autoradiographs of Southern blots of PCR products (15 cycles) of mouse brain and skeletal muscle dystrophin mRNA which had been co-amplified with L-PK transcript as an internal standard. The blot was hybridized with an internal specific oligonucleotide labelled by kination. c, As b, but obtained after 30 cycles of amplification. To prevent diffusion of the strong signal from the muscle-type transcript in muscle after the 72-h exposure, the corresponding lane has been cut out. METHODS. Cells were derived from brains of random-bred Swiss mice. Glial cells were from newborn mouse cerebral hemispheres. Culture conditions and cell-type characterization have been described⁷. Ninety-five per cent of the cells were identified as type-1 astrocytes; neither neurons nor oligodendrocytes were detected in multiple screenings. Cultures of neuronal cells were set up from single-cell suspension of fetal brains at 15 days of gestation^{6,12}. Cultures consisted predominantly of neurons (>95%), identified by surface labelling with tetanus toxin and intracellular labelling with antibodies to γ -enolase or neurofilament proteins. In older cultures, 1-5% of cells were astrocytes. Co-amplification was performed as follows: to 5 μ g total RNA of each specimen, an exogenous sample of reference RNA (0.2 μ g total rat liver RNA) was added as a source of internal standard mRNA. cDNA was synthesized by extension of both dystrophin-specific primer 2C and L-PK-specific primer⁴. After this first step, each sample was divided into two aliquots in which co-amplification was carried out with 1B and 2C primers for the brain transcripts (slots B), and with 1M and 2C for the skeletal muscle transcripts (slots M); L-PK primers were included in each vial for co-amplification. After 15 cycles, half of the reaction volume was removed from each vial and the remainder was exposed to 15 further cycles of amplification. Each sample was then Southern blotted and hybridized first with the dystrophin probe, and then stripped and rehybridized with the L-PK probe. Relative quantitation was achieved by densitometry of autoradiographs exposed after variable times, using L-PK as a standard⁴.

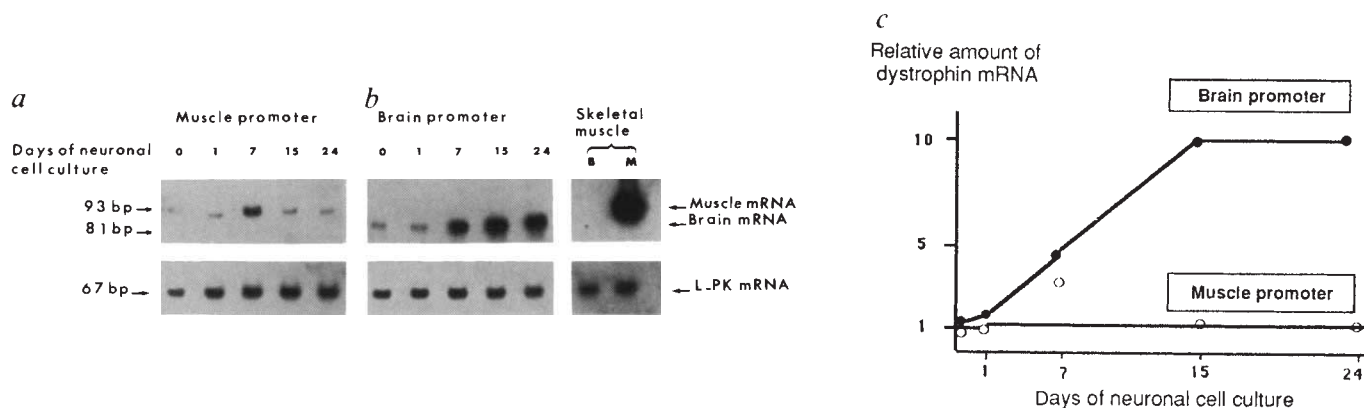


FIG. 2 Study of the brain-type and muscle-type dystrophin transcripts during neuronal maturation. *a*, Southern blots of PCR-amplified muscle-type transcripts (15 cycles) from mouse cultured neurons (top) co-amplified with L-PK transcripts used as internal standard (bottom). *b*, Southern blots of PCR-amplified brain-type transcripts (15 cycles) from mouse cultured

neurons (top) co-amplified with L-PK transcripts used as internal standard (bottom). *c*, Relative amounts of brain-type and muscle-type transcripts of the dystrophin gene as a function of time of neuronal maturation. Methods are described in the legend to Fig. 1.

in glial cells, as in skeletal muscle, only the muscle promoter is used. In neurons, the brain-type mRNA is more abundant than the muscle-type mRNA, whereas it was not detected in either glial cells or skeletal muscle. After 30 cycles of amplification, however, minute amounts of transcript driven by the brain-type promoter are detectable in muscle and glial cells (Fig. 1c).

We also investigated the relative amounts of brain-specific and muscle-specific expression of the dystrophin mRNA in the course of *in vitro* maturation of neuronal cells from fetal mouse brain (see Fig. 2 legend). Figure 2 shows that the amount of brain-specific transcript increases steadily, reaching a plateau at day 14, with a total increment of ~ 10 . By contrast, the muscle-specific transcript does not vary significantly, and after the fifteenth day of culture represents $\sim 10\%$ of the brain-specific transcript (Fig. 2).

Second to myogenic tissues, the central nervous system has the highest level of expression of the dystrophin gene, demonstrated by the levels of both mRNA^{8,9} and protein¹⁰. It has already been suggested that in brain the dystrophin expression is neuronal in origin¹⁰, and, in primary cultures of mouse brain cells, we find it is greater in neuronal than in glial cells⁴. As transcription of the first exon of brain dystrophin is driven by a specific promoter^{2,3}, we attempted to define the type of promoter used in neuronal and glial cells. Our results indicate that the brain promoter is active only in neurons, whereas the muscle promoter is used in skeletal muscle, in glial and in neuronal cells. A weak contamination by astrocytes ($\sim 1\text{--}5\%$), might contribute to the finding of some muscle-type transcript in cultured neurons. The muscle-type promoter is also active in cardiac and smooth muscle (data not shown). The trace amounts of brain-promoted transcripts that we find in muscle and glial cells after 30 cycles are probably attributable to illegitimate transcription¹¹ and are comparable to the levels of muscle-type transcript that we detect in cultured fibroblasts (Fig. 1c). We believe that this would account for an earlier discovery³ of brain-specific transcript in muscle. In every cell type that we investigated, apart from neurons, the muscle promoter is stronger and more leaky than the brain promoter. This could explain why the illegitimate transcript found in fibroblasts is mainly transcribed from the muscle-type promoter. The increase that we observed during maturation of neurons in culture and in developing mouse brain (data not shown) indicates that brain-type dystrophin mRNA and protein could be neuron-specific developmental markers, like the voltage-gated sodium channel¹² and tubulin¹³. The sub-cellular location and function of dystrophin in neurons must be determined before any relation can be established between

expression of this protein in brain and the mental retardation found in some Duchenne muscular dystrophy patients. □

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Role of self-peptides in positively selecting the T-cell repertoire

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THE fate of an immature thymocyte is determined by the specificity of its $\alpha\beta$ T-cell receptor. Only cells expressing receptors that interact with sufficient affinity with major histocompatibility complex (MHC) molecules expressed on thymus epithelial cells are positively selected and go on to mature and seed the peripheral lymphoid organs^{1–4}. The H-2K^b class-I MHC molecule positively selects for the maturation of cytotoxic T lymphocytes that will respond in the periphery to H-2K^b cells presenting a foreign peptide. We have now analysed the ability of variant H-2K^b molecules to positively select T-cells that respond to H-2K^b with ovalbumin. Our results indicate that self-peptides, presented in the groove of the class-I molecule on thymus epithelial cells, are critically involved in positive selection of the T-cell repertoire. Furthermore, the ability of four different H-2K^b variants to select this response in the thymus correlates with their ability to present the ovalbumin peptide, indicating that a self-peptide mimic of the foreign peptide could be involved in positive selection.

The structure of a class-I MHC molecule reveals a likely peptide-binding groove composed of a 'floor' of antiparallel β -strands and 'walls' of two long α -helices⁵. It has been proposed that during MHC-restricted recognition of foreign peptide the T-cell receptor makes contact with the outer face of the α -helices and the peptide lying between them⁶⁻⁸. Because the selection of the T-cell repertoire occurs during T-cell differentiation in the thymus in the absence of foreign peptide, we speculated that the outer face of the α -helices would be particularly important in positive selection and the peptide-binding groove would exert less influence. To determine whether this is so, we compared the ability of several H-2K^b variant MHC molecules to select a H-2K^b-restricted repertoire. We were most interested in two H-2K^b mutants, H-2K^{bm8} (changes at positions 22, 23, 24 and 30) and H-2K^{bm5} (changes at position 116), with substitutions only in the β -strands forming the floor of the groove^{9,10}. These changes are probably not detectable on the exterior of the molecule, and in fact these variants are serologically indistinguishable from wild-type H-2K^b with many antibodies^{9,11}. Their effects on T-cell recognition are likely to be due to changes in peptide binding¹⁰. Comparison of the structure of HLA-A2 with that of a second class-I molecule, HLA-Aw68, with 10 residue differences in positions lining the floor and walls of the groove, shows that each substitution causes only very local structural changes¹². The backbone structure of the two molecules is the same, and unaltered residues remain essentially unperturbed. Two other H-2K^b mutants, H-2K^{bm1} (changes at positions 152, 155 and 156) and H-2K^{bm3} (changes of positions 77 and 89), have substitutions on the α -helices that locally affect serological sites⁹ as well as point into the groove and affect peptide presentation¹⁰.

C57BL/6 (B6, H-2^b) mice are able to mount an ovalbumin (OVA)-specific cytotoxic T lymphocyte (CTL) response when they are immunized with syngeneic cells transfected with an OVA vector or with spleen cells carrying OVA¹³. All the CTL activity is restricted by the H-2K^b class-I molecule and specific for the OVA₂₅₃₋₂₇₆ region (containing residues 253-276 of OVA). The response is heterogeneous, because analysis of clones and long-term lines has shown that T-cell receptor V β 5, V β 8 as well as other V β segments, are represented in the CTL response. To study the influence of changes in the class-I molecule on the selection of the H-2K^b-restricted repertoire, we injected bone marrow cells from (B6.PL-Thy-1^a × H-2K^b-mutant)F₁ mice into lethally irradiated B6 or H-2K^b-mutant mice. After 8-12 weeks, when bone marrow-derived T-cells had matured in the irradiated hosts, we primed the mice with OVA on H-2^b cells. We boosted spleen cells from these mice *in vitro* with an EL4 transfectant synthesizing OVA (E.G7-OVA) or with allogeneic H-2^d spleen cells, and measured CTL activity 5 days later. We typed the H-2^d-specific effector cells for their expression of Thy-1.1 (indicating bone-marrow origin) or Thy-1.2 only (indicating host

TABLE 1 Ability of H-2K^b-mutant MHC molecules to select the CTL response to OVA assayed in [F₁ → parent] radiation chimaeras

Radiation chimaeras	OVA-specific response		Alloreactive response		
	% Specific lysis of targets EL4	% Specific lysis of targets E.G7	% Specific lysis of targets EL4	% Specific lysis of targets P815	% Donor* T-cells
Expt 1					
(B6.PL × bm1)F ₁ → bm1	3/0	0/1	4/2	48/30	92
(B6.PL × bm3)F ₁ → bm3	10/6	41/27	2/0	41/18	91
(B6.PL × bm5)F ₁ → bm5	8/4	37/27	7/4	47/24	83
(B6.PL × bm8)F ₁ → bm8	6/3	10/10	6/3	43/24	74
Expt 2					
(B6.PL × bm5)F ₁ → B6	8/3	56/47	3/4	70/49	83
(B6.PL × bm5)F ₁ → bm5	7/4	56/42	6/3	77/58	85
(B6.PL × bm8)F ₁ → B6	2/3	58/54	4/2	65/47	78
(B6.PL × bm8)F ₁ → bm8	10/6	7/8	6/4	69/54	70

Radiation chimaeras were made by injecting 10⁷ F₁ bone marrow cells depleted of T-cells by antibody-with-complement treatment into lethally irradiated (950 rad) female recipients of the indicated type, [F₁ bone marrow → irradiated host]^{1,2}. The chimaeras were maintained on antibiotic water and were primed after 8-12 weeks by intravenous injection of 2.5 × 10⁷ irradiated spleen cells containing OVA as described previously²². In expt 1, spleen cells syngeneic with the marrow donor were used to prime, and in expt 2, B6.PL spleen cells were used. Seven days after priming, spleen cells from the chimaeras were stimulated in culture with irradiated (12,000 rad) E.G7-OVA cells¹³ to boost the OVA-specific response, or with irradiated (3,000 rad) BALB/c (H-2^d) spleen cells to induce an alloreactive response. After 5 days in culture, CTL activity was assayed in a 3-hr ⁵¹Cr-release assay. Percentage specific lysis at two effector-to-target ratios are shown: 6:1 and 2:1 (expt 1) and 100:1 and 33:1 (expt 2).

* CTL activity from the cultures stimulated with allogeneic BALB/c (H-2^d) spleen cells assayed on P815 (H-2^d) targets was typed for sensitivity to anti-Thy-1.1 antibody (19E12)²³ and anti-Thy-1.2 antibody (13.4)²⁴ with complement as described to determine the fraction of donor-derived (Thy-1.1 × Thy-1.2) effector cells. In brief, effector cells were treated with complement alone or with one or both antibodies with complement for 45 min at 37 °C before assaying on ⁵¹Cr-P815 target cells. Controls with Thy-1.1 and Thy-1.2 CTL from B6.PL (Thy-1.1) and B6 (Thy-1.2) mice showed that both antibodies were Thy-1 allele-specific in their activity.

origin). Cells that matured in an irradiated H-2K^{bm1} or H-2K^{bm8} host were not able to respond to H-2K^b with OVA (Table 1). By contrast, T-cells that matured in a wild-type H-2K^b, H-2K^{bm3} or H-2K^{bm5} host responded to OVA measured as the specific lysis of E.G7-OVA target cells. Spleen cells from all groups could respond to H-2^d cells in primary mixed lymphocyte culture to generate CTL that lyse P815 (H-2^d) target cells and 70-92% of this alloreactive CTL activity was sensitive to anti-Thy-1.1 antibody and complement, and therefore due to CTL of bone marrow origin. The failure of the [B6.PL × bm8 → bm8] chimaeras to respond to OVA implies that peptide is important in repertoire selection.

The nonresponsiveness of the [B6.PL × bm8 → bm8] and [B6.PL × bm1 → bm1] chimaeras could possibly be due to tolerance. This is unlikely because, as shown in Table 2, (B6.PL × bm8)F₁ and (B6.PL × bm1)F₁ mice responded to OVA with H-2K^b. But the homozygous mutant mice B6.C-H-2^{bm1} and B6.C-H-2^{bm8} are themselves nonresponsive to OVA. The other

FIG. 1 Presentation of the OVA₂₅₃₋₂₇₆ peptide to B6 CTL by cells expressing H-2K^b-mutant molecules. Spleen cells from B6 mice that had been primed with OVA on syngeneic cells were boosted in culture for 5 days with irradiated E.G7-OVA cells and assayed for lysis of various targets that had been pulsed with medium or with the OVA₂₅₃₋₂₇₆ peptide. Target cells were SP2 (BALB/c, H-2^d myeloma) or hybrids formed by polyethylene glycol-mediated fusion of SP2 with spleen-derived lipopolysaccharide blasts from B6 or H-2K^b-mutant mice. S.B6.2 is a hybrid with B6; S.bm1.3 is a hybrid with bm1, and so on. The hybrid lines all express H-2D^b and the expected wild-type or mutant H-2K^b molecule as shown by serological and T-cell typing. Target cells were incubated with synthetic OVA₂₅₃₋₂₇₆ peptide, 100 µg ml⁻¹ for 1 h at 37 °C and washed before the 3-hr ⁵¹Cr-release assay. ●, OVA₂₅₃₋₂₇₆; ○, no antigen.

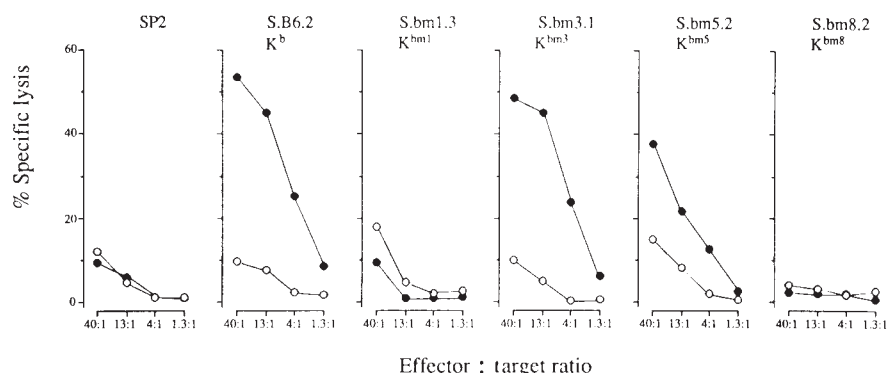


TABLE 2 Failure to respond to OVA in the [$F_1 \rightarrow$ parent] radiation chimaeras is not due to tolerance

	Responder	% Specific lysis on targets:	
		E.G7-OVA	EL4
Expt 1	B6	60/41	17/9
	(B6 $\times$ bm1) F_1	47/30	18/7
	(B6 $\times$ bm3) F_1	66/53	25/8
	(B6 $\times$ bm5) F_1	58/46	14/6
	(B6 $\times$ bm8) F_1	53/36	9/8
Expt 2	B6	60/53	0/0
	bm1	4/2	2/2
	bm3	41/25	3/0
	bm5	54/39	3/1
	bm8	12/6	23/15

The indicated responder mice were immunized by intravenous injection of 2.5×10^7 irradiated (1,000 rad) syngeneic spleen cells that had been 'loaded' with OVA¹³. Seven days later, spleen cells from the primed animals were re-stimulated in culture with syngeneic spleen cells that had been incubated with a CNBr-digest of OVA, 100 μ g ml⁻¹, and washed. The CNBr fragments of OVA include the H-2K^b-restricted targeting peptide. CTL activity was measured 5 days later and is expressed as % specific lysis at effector-to-target ratios of 50:1 and 17:1. The ability of the CTL to lyse E.G7-OVA targets correlated with their ability to lyse syngeneic H-2K^b-mutant targets in the presence of the OVA peptide.

TABLE 3 [$F_1 \rightarrow$ parent] radiation chimaeras contain H-2K^b-expressing antigen-presenting cells

Responder spleen cells	Stimulator spleen cells	Anti-H-2K ^b -specific response (% specific ⁵¹ Cr release)	
		P815	EL4
bm1	B6	13/4	58/37
	(B6.PL $\times$ bm1) F_1	16/6	64/48
	(B6.PL $\times$ bm1) $F_1 \rightarrow$ bm1	12/8	55/39
bm8	B6	10/1	67/62
	(B6.PL $\times$ bm8) F_1	8/1	64/47
	(B6.PL $\times$ bm8) $F_1 \rightarrow$ bm8	8/3	67/47

Responder spleen cells from naive B6.C-H-2^{bm1} (bm1) or B6.C-H-2^{bm8} (bm8) mice were cultured *in vitro* for 5 days with an equal number of 3,000 rad spleen cells from stimulator mice as shown. CTL activity was assayed 5 days later on P815 (H-2^d) and EL4 (H-2^b) target cells at effector-to-target ratios of 48:1 and 16:1.

mutant mice, B6.C-H-2^{bm3} and B6.C-H-2^{bm5}, mounted a CTL response to OVA which cross-reacts on OVA with H-2K^b (Table 2). Therefore we saw a correlation between the ability of four H-2K^b mutants to make a CTL response to OVA and their ability to select the OVA-with-H-2K^b repertoire in radiation chimaeras. To study this more closely, we analysed the ability of the H-2K^b variants to present the H-2K^b restricted OVA peptide *in vitro*. A heterogeneous CTL line from B6 mice specific for H-2K^b with OVA₂₅₃₋₂₇₆ was tested for lysis of target cells expressing different class-I molecules. CTL did not lyse SP2 (H-2^d) cells in the presence or absence of the OVA peptide, but a hybrid cell line, S.B6.2 (SP2 fused with B6 cells), was lysed in a peptide-specific way (Fig. 1). Target cells expressing H-2K^{bm1} or H-2K^{bm8} however, were not recognized by this CTL line in the presence of peptide, whereas H-2K^{bm3} and H-2K^{bm5} cells were peptide-specific targets. In other experiments we have generated eight independent CTL clones from B6 mice specific for H-2K^b with OVA₂₅₃₋₂₇₆, none of which recognizes H-2K^{bm1} or H-2K^{bm8} presenting cells.

The inability of cells derived from H-2K^{bm1} or H-2K^{bm8} mice to present the OVA peptide indicates a trivial explanation for

the failure of such mice to select the response in the [$F_1 \rightarrow$ parent] chimaeras. So, although we typed the alloreactive CTL as >70% of bone-marrow donor origin, the cells in the chimaeras presenting OVA to resting CTL could still have been of host origin. If this were the case, then [B6.PL $\times$ bm1 $\rightarrow$ bm1] and [dB6.PL $\times$ bm8 $\rightarrow$ bm8] chimaeras would have inappropriate presenting cells for the H-2K^b-with-OVA response. This explanation is unlikely to be true for two reasons. First, previous work indicates that antigen-presenting cells turn over rapidly after irradiation¹⁴, and second, we always injected the mice with immunogenic cells expressing H-2K^b. Nevertheless, we tested the hypothesis by assaying the ability of spleen cells from these chimaeras to stimulate anti-H-2K^b CTL. Splenocytes from [B6.PL $\times$ bm1 $\rightarrow$ bm1] and [B6.PL $\times$ bm8 $\rightarrow$ bm8] radiation chimaeras were as effective in stimulating an H-2K^b-specific CTL response as were cells from normal F_1 animals (Table 3). We conclude that the nonresponsive chimaeras had appropriate H-2K^b-expressing presenting cells.

The H-2K^{bm8} molecule differs from wild-type H-2K^b in three positions in a β -strand in the floor of the peptide-binding site. Two of these, at positions 22 and 24, point up into the site, whereas position 23 points down¹⁰. The inability of H-2K^{bm8} to select the heterogeneous response to H-2K^b with OVA₂₅₃₋₂₇₆ indicates very strongly that self-peptides associated with the class-I molecules of thymic epithelial cells play a critical part in selecting the T-cell repertoire. The ability of variant H-2K^b molecules to select is antigen-specific, because [B6.PL $\times$ bm8 $\rightarrow$ bm8] chimaeras are responders to VSV nucleocapsid protein with H-2K^b, meaning that H-2K^{bm8} does select for this H-2K^b-restricted response (data not shown). The idea that tissue-specific self-peptides^{15,16} or erroneous self-peptides¹⁷ are involved in positive selection has been suggested previously, and in some cases background genes (that is, not the restriction element) influence selection¹⁸⁻²⁰. How the correlation that we observed between the ability of class-I molecules to present a foreign peptide and its ability to select the appropriate repertoire in the thymus comes about is difficult to understand and may not strictly apply for all antigens²¹. The restriction element would be expected to select sites on the antigen which interact with the MHC (agretopes), whereas the T-cell receptor would be expected to interact with other regions of the antigen (epitopes). But T-cells that respond to a foreign peptide binding only to one side of the class-I groove and obscuring that part of the MHC molecule, may have to make many important receptor contacts with the opposite side of the MHC molecule. Thymic selection of such a T-cell receptor repertoire would depend on thymic MHC presenting peptides in a similar way. □

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Origin of Thy-1⁺ dendritic epidermal cells of adult mice from fetal thymic precursors

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THE skin of mice contains dendritic epidermal cells carrying the Thy-1 antigen (Thy-1⁺ dEC) which express antigen receptors composed of the T-cell antigen receptor (TCR) γ - and δ -chains¹⁻⁵. Although the role of the thymus in the generation of most T cells is well established⁶, the involvement of the thymus in the generation of Thy-1⁺ dEC is not clear. Because bone marrow cells can give rise to Thy-1⁺ dEC in chimaeric mice⁷ and Thy-1⁺ dEC are detected in the skin of athymic nude mice⁸, it has been proposed that Thy-1⁺ dEC arise continuously from bone marrow precursors by a thymus-independent mechanism⁹. But it has recently been determined that Thy-1⁺ dEC in nude mice do not express TCR at the cell surface, and that the γ - and δ -chain genes are in germ-line configuration¹⁰, leaving the role of the thymus in the generation of Thy-1⁺ dEC uncertain. Most Thy-1⁺ dEC in all normal mouse strains examined express TCR containing the V γ 3 gene product¹¹. This V gene segment is expressed on the first wave of TCR-expressing cells to emerge during fetal development, and in adult mice is detectable only on cells in the epidermis¹². In addition to use of this 'fetal' V γ segment^{1,2,13,14}, other features of the Thy-1⁺ dEC TCR genes, including absence or minimal presence of non-germ-line-encoded nucleotides at the junctions and use of a single D element in the rearranged δ -chain gene^{15,16} are typical of rearrangements found in fetal, and not adult, thymus^{17,18}. Here we demonstrate that precursors that are present only in the fetal thymus give rise to Thy-1⁺ dEC in the skin of adult mice.

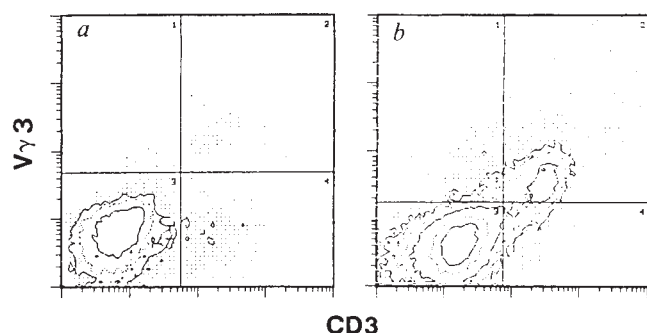


FIG. 1 Analysis of Thy-1⁺ cells in nude mouse skin after fetal thymus implant. Fetal thymic lobes were removed from fetal mice on day 14 of gestation and four lobes engrafted under the arm of each nude mouse using a trocar. At the time of analysis, the engrafted thymic lobes were removed for analysis and skin used only from mice that had demonstrated donor thymus engraftment. Epidermal sheets were removed from *a*, control nude mouse skin and *b*, skin 2 months after implantation of day-14 fetal thymic lobes dissociated by digestion with trypsin, and cells isolated by ficoll-hypaque density gradient centrifugation²¹. Interface cells were incubated with biotin-anti-Thy-1 (monoclonal antibody HK2.1)²², arsenyl (ARS)-derivatized anti-CD3 (monoclonal antibody 500A2)²³, and fluorescein isothiocyanate (FITC)-anti-V γ 3 (monoclonal antibody 536)¹¹ for 1 h at 4 °C. After washing, allophycocyanin-avidin and phycoerythrin-anti-ARS were added for an additional hour at 4 °C. Electronic gates were set to resolve Thy-1⁺ cells and data displayed as two-colour contour plots of CD3- and V γ 3-specific monoclonal antibody binding. Cells were analysed on a FACS 440 system with an argon ion laser (488 nm) and a helium neon laser (633 nm) (Becton Dickinson). Data analysis was performed using the Consort 30 system (Becton Dickinson).

The Thy-1⁺ dEC population in athymic nude mouse skin contains virtually no V γ 3⁺ or CD3⁺ cells (Fig. 1*a*). This is not by itself evidence for thymic involvement in generation of these cells, because the mice have an obvious skin defect which could interfere with the development of homing of the cells. But implantation of day-14 fetal thymic lobes from normal mice results in the appearance of a large population of V γ 3⁺ CD3⁺ Thy-1⁺ dEC (Fig. 1*b*). This result demonstrates that the skin of nude mice is not refractory to the appearance of V γ 3⁺ CD3⁺ Thy-1⁺ dEC, and that the presence of a fetal thymus is sufficient to give rise to this population. That the TCR- Thy-1⁺ population remains indicates that the thymus does not indirectly support the development of the resident Thy-1⁺ dEC, but either contains precursor cells or is capable of supporting the development of host-derived bone marrow cells, or both.

To examine whether the thymus contains cells that directly seed the skin, we grafted thymic lobes from Thy-1.1⁺ mice into newborn Thy-1.2⁺ mice. Engraftment with thymic lobes from day-14 fetal mice resulted in the appearance of donor-type Thy-1⁺ dEC in the skin (Fig. 2*a*). The day-14 thymus contained ~5% V γ 3⁺ cells, indicating that these cells can emigrate from the grafted thymus to the host skin and give rise to the donor-type dEC. Engraftment with thymic lobes from newborn mice (Fig. 2*c*) did not result in the appearance of donor-type Thy-1⁺ dEC in the skin of recipients, perhaps because V γ 3⁺ cells are not detectable in the thymus after day 18 of gestation¹².

The latter result indicates that the adult thymus does not support the generation of V γ 3⁺ Thy-1⁺ dEC precursors. To examine this possibility, we exploited the fact that repopulation of the adult thymus after sublethal irradiation recapitulates in many respects the events of normal ontogeny¹⁹. Thymocytes of irradiated mice were examined daily for 23 days for expression of antigens CD4 and CD8, and V γ 3. At no time were V γ 3⁺ cells detected, even when the analysis was enhanced for detection of $\gamma\delta$ TCR⁺ cells by gating on the CD4⁺ CD8⁺ population

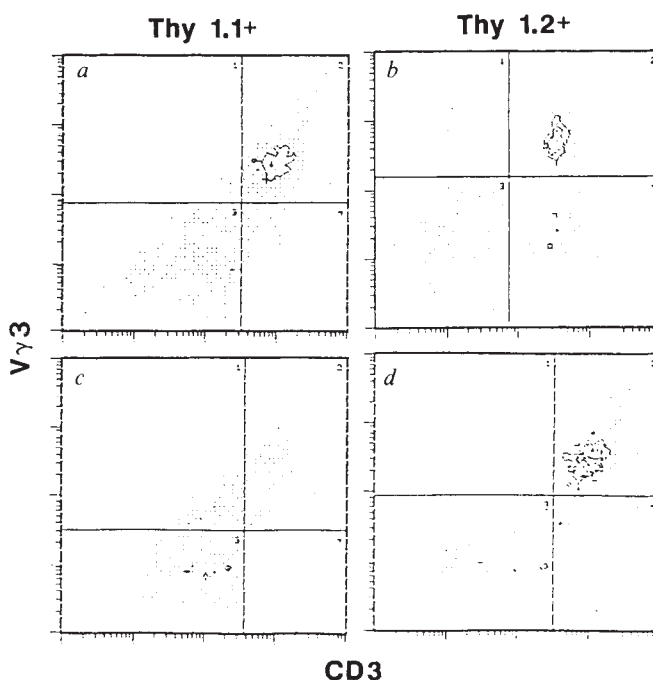


FIG. 2 Analysis of Thy-1⁺ dEC from skin of normal mice after implantation of fetal or newborn congenic thymus lobes. Day-14 fetal (*a, b*) or newborn day-2 (*c, d*) thymic lobes from Thy-1.1⁺ C57BL/6 congenic mice were engrafted into newborn day-4 C57BL/6 (Thy-1.2⁺) mice as described in Fig. 1. Skin was analysed 2 months after thymic engraftment. The presence of Thy-1.1⁺ donor (*a, c*) and Thy-1.2⁺ host (*b, d*) dEC is shown by multicolour immunofluorescence analysis with CD3- and V γ 3-specific monoclonal antibodies as described in Fig. 1.

FIG. 3 T-cell receptor expression on recovering thymocytes in sublethally irradiated mice. Six-week-old C57BL/6 mice received 750 rad from a ^{51}Cs irradiator. Thymocytes were examined daily by multicolour immunofluorescence for expression of antigens CD4, CD8 and CD3, and $V\gamma 3$. Analysis was performed using biotin-anti-CD8 (monoclonal antibody 3.155)²², biotin-anti-CD4 (monoclonal antibody GK1.5)²⁴, ARS-anti-CD3, and FITC-anti- $V\gamma 3$ as described in Fig. 1. Electronic gates were set to resolve $\text{CD4}^- \text{CD8}^-$ cells (allophycocyanin-avidin-negative population) and data is shown for $\text{CD4}^- \text{CD8}^-$ thymocytes obtained from mice on days 3, 5, 8, 10 and 12 after irradiation.

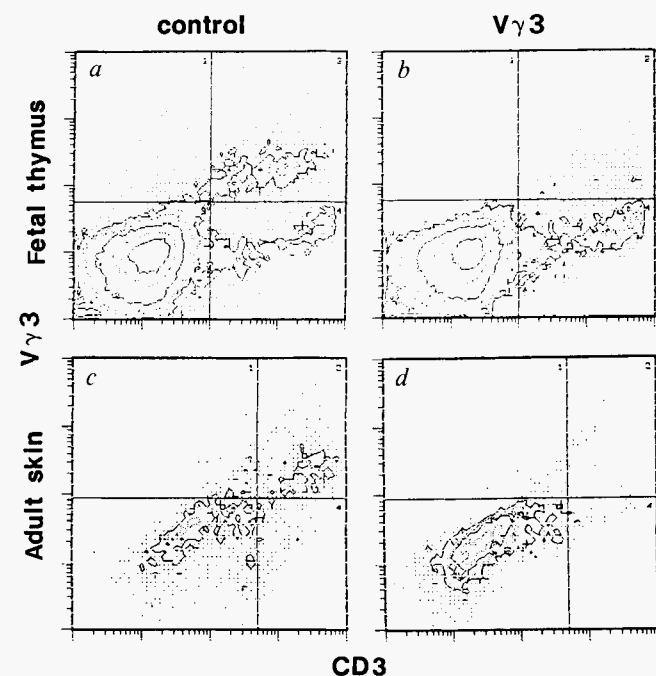
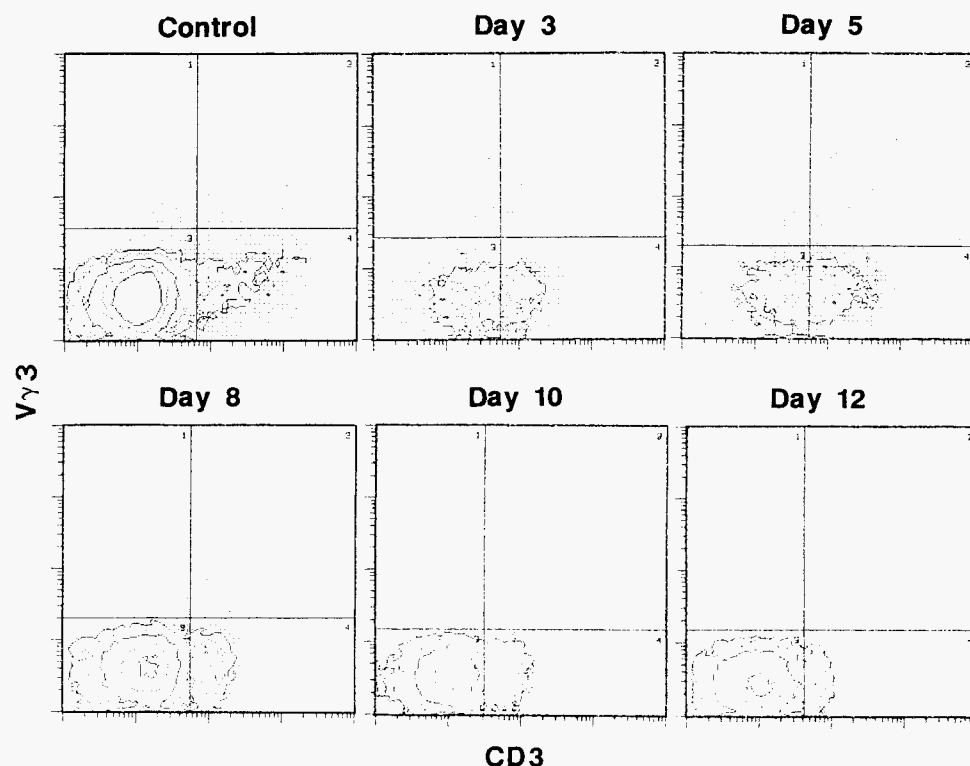


FIG. 4 Analysis of mice treated with antibody *in utero*. Pregnant BALB/c mice were treated intravenously with 1 mg control (145-10D8) or anti- $V\gamma 3$ (536) monoclonal antibody daily from day 10 of gestation until birth (day 20). Fetal thymocytes from *a*, control and *b*, anti- $V\gamma 3$ monoclonal antibody-treated animals were examined on day 16 of gestation for the presence of $V\gamma 3^+$ cells. Skin epidermal cells were analysed 4 months after birth of pups from *c*, control and *d*, anti- $V\gamma 3$ monoclonal antibody-treated females. No residual injected monoclonal antibody could be detected in the serum of the pups at the time of analysis. The analysis was performed as described for Fig. 1, except that gates were not set to resolve Thy-1^+ cells as no Thy-1^+ cells could be detected in skin from the anti- $V\gamma 3$ -treated mice.

(Fig. 3). These results indicate that the ability of the thymus to generate the wave of $V\gamma 3^+$ cells is lost with maturation.

If generation of $V\gamma 3^+$ cells is restricted to an early period of ontogeny, ablation of these cells as they appear might be expected to result in the absence of Thy-1^+ dEC in mature mice. To test this possibility, we exposed embryos to anti- $V\gamma 3$ antibody or a control hamster antibody *in utero* by daily injections of antibody from day 10 of gestation until parturition (day 20). Analysis of day-16 fetal thymocytes indicated that $V\gamma 3^+$ cells were absent in mice treated with anti- $V\gamma 3$ monoclonal antibody (Fig. 4*b*). Four months after birth and the end of antibody treatment, we examined the skin of the mice for the presence of Thy-1^+ dEC. We were unable to detect residual antibody in the serum of the mice at this time. The skin of control mice contained abundant $V\gamma 3^+$ Thy-1^+ dEC (Fig. 4*c*), whereas these cells were absent from the skin of mice that had been treated with anti- $V\gamma 3$ antibody (Fig. 4*d*). These results indicate that generation of Thy-1^+ dEC precursors is restricted to fetal stages of development.

Taken together, these data provide support for the notion that the first wave of TCR^+ thymocytes that appear during ontogeny emigrate from the thymus and give rise to T cells found in epidermis of adult mice. The levels of TCR^+ Thy-1^+ dEC in the skin may be maintained by proliferation of these early fetal emigrants, but unlike other T cells, cannot be replaced by generation of new precursors in the adult animal. An implication of this finding is that the antigen receptor repertoire of descendants of fetal precursors may be limited by the extent to which the diversity-generating mechanisms operate when precursor TCR genes rearrange. In addition to the rearrangement and expression of $V\gamma$ - and $V\delta$ -genes proceeding in an ordered manner^{13,17,18}, early fetal δ -chain genes are most often restricted to using a single *D* element, and non-germ-line-encoded nucleotides are often absent from both γ - and δ -chain genes^{17,18}. The net effect of these developmentally regulated aspects of TCR rearrangement is to severely restrict the TCR repertoire available to Thy-1^+ dEC in the adult skin^{15,16}. We have proposed that the

restricted repertoire of the cells generated early in ontogeny makes them suitable to perform trauma signal surveillance for self-products associated with cellular damage rather than surveillance for foreign antigens^{15,16,20}, a possibility that is now being investigated. The relationship between the second and later waves of TCR-bearing cells appearing during thymic development and other epithelium-associated T cells, and the TCR repertoire and biological roles of these cells, remains to be established. □

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A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection

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RHINOVIRUSES belong to the picornavirus family and cause about 50% of common colds¹. Most rhinoviruses and some coxsackie viruses share a common receptor on human cells. The glycoprotein intercellular adhesion molecule-1 (ICAM-1) has recently been identified as the cellular receptor for the subgroup of rhinoviruses known as the major groups^{2–4}. ICAM-1 is a member of the immunoglobulin supergene family and is a ligand for lymphocyte function-associated antigen-1 (LFA-1)^{5–7}; these ICAM-1/LFA-1 interactions are critical to many cell adhesion processes involved in the immunological response^{8–11}. Because anti-ICAM-1 antibodies can block binding of major-group rhinoviruses to cells, we considered that antagonism of virus-receptor interaction might be a way of preventing rhinovirus infection. We have constructed and purified a soluble form of the ICAM-1 molecule, which is normally membrane-bound, and demonstrated that it is a potent and specific inhibitor of rhinovirus infection.

The ICAM-1 molecule is composed of five immunoglobulin-like extracellular domains, a hydrophobic transmembrane domain, and short cytoplasmic domain. To produce large amounts of soluble ICAM-1 (sICAM-1), we used site-directed mutagenesis to introduce an in-frame translational stop codon at the predicted extracellular boundary of the transmembrane domain, effectively deleting the transmembrane and cytoplasmic domains of the protein. After expression and analysis of the resulting truncated gene product in transient expression assays, a stable line of ICAM-1-secreting Chinese hamster ovary (CHO) cells was produced. The sICAM-1 gene was physically linked to the hamster dihydrofolate reductase gene in an SV40-based expression vector and then transfected into CHO cells. After selection in methotrexate, clones secreting sICAM-1 were identified by enzyme-linked immunosorbent assay. Two further rounds of gene amplification gave a cell line (designated CHO118A) that secreted sICAM-1 into the culture supernate to a concentration of about 1 $\mu\text{g ml}^{-1}$.

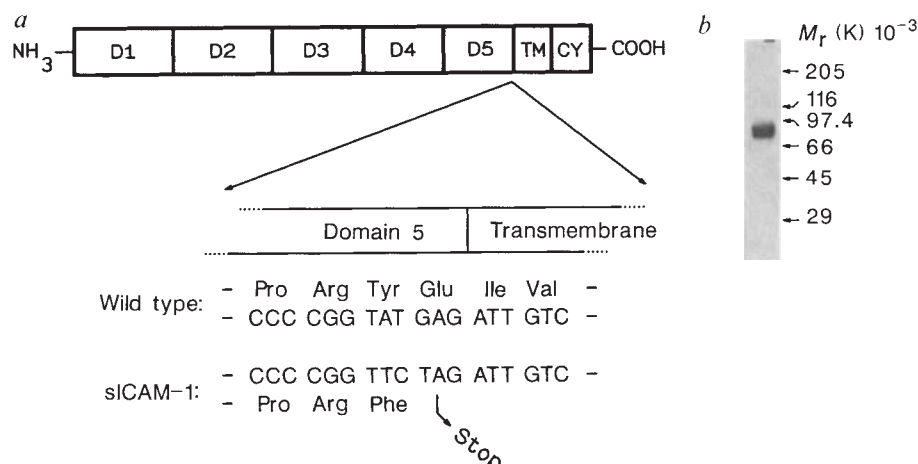
We purified milligram quantities of sICAM-1 to >95% purity from these CHO118A cell-culture supernates by immunoaffinity chromatography with the anti-ICAM-1 monoclonal antibody R6.5. Purified sICAM-1 has an apparent relative molecular mass (M_r) of 82,000 (82K), which is consistent with the predicted size of a molecule containing all five extracellular immunoglobulin-like domains. The purified sICAM-1 binds to three distinct monoclonal antibodies raised against membrane-bound ICAM-1 (mICAM-1): RR1/1, R6.5, and CL203 (data not shown). These antibodies bind to topographically distinct sites, as assayed by competitive binding (data not shown), and because they bind to sICAM-1, we infer that the overall conformation of native mICAM-1 is maintained.

We next tested whether sICAM-1 could inhibit rhinovirus infection in a quantitative *in vitro* assay for virus cytopathic effect (CPE)³. As shown in Fig. 2, sICAM-1 is a potent inhibitor of human rhinovirus strain 54 (HRV54), a major-group virus: sICAM-1 at 1 $\mu\text{g ml}^{-1}$ (~18 nM) significantly inhibits CPE (by ~50%), and there is >90% inhibition at 10 $\mu\text{g ml}^{-1}$. By contrast, a control derived from column fractions adjoining the sICAM-1 peak has no effect.

The specificity of inhibition by sICAM-1 was investigated using rhinoviruses from both the major and minor subgroups, other picornaviruses, and herpes simplex virus type-1 (HSV-1), an unrelated enveloped DNA virus. As shown in Fig. 3, sICAM-1 inhibits HRV54, but has no significant effect on HRV2, a minor-group strain that does not use ICAM-1 as its cellular receptor. In addition, sICAM-1 inhibits infection by coxsackie A13, another picornavirus using the major-group receptor¹². By contrast, sICAM-1 does not inhibit poliovirus, coxsackie B1 (picornaviruses that do not bind to cells through ICAM-1) or HSV-1. The specificity of virus inhibition indicates that sICAM-1 prevents infection not as a result of generalized effects on the cell's ability to support viral replication, but rather by inhibition of virion binding, as we expected. To confirm this directly, we measured the effect of sICAM-1 on virus binding to cells using [³⁵S]methionine-labelled virus and found that the sICAM-1 inhibition of HRV14 (from the major rhinovirus subgroup) binding is dose-dependent, whereas the control has no effect (Fig. 4). As shown previously³, the positive control anti-ICAM-1 antibody R6.5 is also effective, whereas the negative control antibody CL203 (which binds to ICAM-1, but does not inhibit its function³) has no significant effect. Note that the virus-binding assay uses a higher concentration of virus than the CPE assay, which could account for the drop in inhibition compared with CPE.

Although sICAM-1 inhibits virion binding, it is unclear how many of the 60 potential binding sites on the virion¹³ need to be occupied by sICAM-1 to block infection efficiently. In addition, the contribution of each of the five immunoglobulin-like domains of sICAM-1 needs to be assessed. Site-specific mutagenesis and truncation indicate that domains D1 and D2

FIG. 1 Generation and purification of a soluble form of ICAM-1. *a*, Construction and expression of a mutant complementary DNA. The protein domain structure of native membrane-bound ICAM-1 is illustrated at the top: the five extracellular immunoglobulin-like domains (D1–D5), the hydrophobic transmembrane domain (TM), and the cytoplasmic tail (CY) are indicated. A soluble form of ICAM-1 was generated by mutation of the codon for Glu 453 to a translational stop codon using oligonucleotide-directed mutagenesis of the wild-type cDNA; the codon for Tyr 452 was mutated to Phe simultaneously (underlined). The plasmid containing this mutant cDNA has been designated pCDsD1-5 (D.E.S., manuscript in preparation). The truncated sequence of sICAM-1 thus differs from wild-type by a single conservative substitution at its carboxyl terminus. *b*, SDS-PAGE analysis of sICAM-1 immunoaffinity purified from culture supernates of transformed CHO118A cells. About 50 ng purified sICAM-1 was electrophoresed under reducing conditions on a 10–15% polyacrylamide gradient gel and visualized by silver staining. Migration of M_r standards is indicated on the right.



METHODS. *a*, The in-frame stop codon was generated using oligonucleotide-directed mutagenesis based on the method of Kunkel¹⁴, as modified by Peterson and Seed¹⁵. The mutation was made with a single-strand uracil-containing template of the ICAM-1 cDNA subcloned into the expression vector CDM8 (pCD1.8) (ref. 7). A mutant oligonucleotide coding for a stop codon and having a unique restriction site was used to prime the second-strand synthesis reaction. After transformation into *E. coli* and confirmation of the mutation by restriction mapping, expression of a secreted form of ICAM-1 was confirmed by transfection into COS cells and analysis of the supernate by enzyme-linked immunosorbent assay (ELISA) (as described in the legend to Fig. 2) and immunoprecipitation. We constructed an expression vector consisting of the hamster dihydrofolate reductase (*DHFR*) gene and the coding region of the mutant ICAM-1 cDNA controlled by the promoter, splice signals and polyadenylation signal from the SV40 early region. The hamster *DHFR* gene was isolated from the plasmid pBR322DHFR (ref. 16) by digestion with *FspI* and *HindIII*, followed by blunt-end ligation into pSV2gpt (ref. 17) cleaved with *BamHI/HindIII*. The mutant sICAM-1 cDNA was isolated by digestion with *NotI*, Klenow fill-in, and digestion with *HindIII*, then ligated

into the expression vector (prepared by digestion with *Apal*, blunting with Klenow, and digestion with *HindIII* to remove the guanine phosphoribosyl transferase gene). The completed vector was then transfected into CHO KI DUX-B11 cells using the calcium phosphate coprecipitation method¹⁸. After two days of growth in non-selective medium, cells were passaged in selective medium containing methotrexate but lacking hypoxanthine and thymidine. Clones were then isolated, subcloned, and tested for sICAM-1 production by ELISA. Colonies secreting the greatest quantity of sICAM-1 were subjected to two rounds of gene amplification by methotrexate at concentrations of 0.05 to 2 μ M. *b*, sICAM-1 was purified from supernates of CHO118A cells by immunoaffinity chromatography with anti-ICAM-1 monoclonal antibody R6.5. R6.5 was covalently coupled to CNBr-activated Sepharose 4B (Pharmacia LKB) to a final concentration of 5 mg ml⁻¹ packed resin. All chromatography steps were at 4 °C; buffers all contained 0.2 U per ml aprotinin and 1 mM phenylmethylsulphonyl fluoride. Filtered supernate (1 litre) containing ~1 mg sICAM-1 was loaded onto a 30-ml column of R6.5-Sepharose at a flow rate of 1 ml min⁻¹. The column was then washed with 300 ml of 10 mM Tris, 0.15 M NaCl, pH 9.0, at a flow rate of 2.5 ml min⁻¹ to remove unbound material. Bound sICAM-1 was eluted with 50 mM triethylamine, 0.15 M NaCl, pH 9.0, at a flow rate of 1 ml min⁻¹. Fractions were immediately neutralized with 1 M Tris, pH 6.0, to 20 mM. sICAM-1-containing fractions were identified by SDS-PAGE (10–15% gradient gels) and silver staining, then concentrated 10-fold in a Centricon-30 microconcentrators (Amicon).

contain the binding site for rhinovirus, and that this site overlaps with the site required for binding to LFA-1, the physiological receptor for ICAM-1 (D.E.S., manuscript in preparation). As large amounts of purified sICAM-1 can be prepared, it should be possible to resolve the three-dimensional structure of the molecule by X-ray crystallography. Finally, we need to establish how sICAM-1 might affect cell adhesion-dependent functions of the immune system.

The anti-rhinoviral activity of sICAM-1 makes it (or a derivative) an antiviral drug candidate. Soluble forms of CD4, the receptor for the human immunodeficiency virus, are now being tested for clinical efficacy against AIDS. Purified sICAM-1 should also be useful in the design and detection of anti-rhinoviral agents. The receptor binding site on the virus is conserved¹³ and its mutation probably restricted: any mutation that destroys binding to ICAM-1 would be lethal unless there

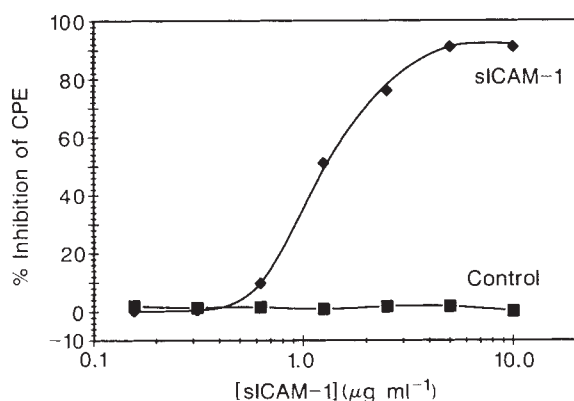


FIG. 2 sICAM-1 inhibits the cytopathic effect (CPE) induced by a major-group rhinovirus. The major-group serotype HRV54 (100 TCID₅₀, where one TCID₅₀ is the concentration required to cause 50% cytopathogenicity³ in tissue culture was plated onto HeLa cells in the presence of sICAM-1 at the concentrations indicated (or an equivalent dilution of a buffer control from the same purification run) and the cytopathic effect determined after 4 days as described³. Data are representative of two experiments.

METHODS. Purified sICAM-1 was assayed for protein using a Bio-Rad kit and frozen in aliquots for use as standards. The concentration of sICAM-1 in samples was determined in a 'sandwich'-type ELISA using these reference standards and two anti-ICAM-1 monoclonal antibodies, R6.5 and R6.1 (ref. 19), which bind to non-overlapping epitopes (S.D.M., unpublished data). R6.1 was bound to 96-well plates (Nunc) by incubating 100 μ l of a 10 μ g ml⁻¹ solution for 1 h at 37 °C. Each of the following steps used 100 μ l reagent incubated at 37 °C for 30 min, followed by washes with phosphate-buffered saline: (1) binding of serial dilutions of reference standard sICAM-1 or unknowns, (2) binding of biotinylated R6.5 (1 μ g ml⁻¹), and (3) binding of horseradish peroxidase-conjugated streptavidin (Zymed). After incubation for 20 min at room temperature with the substrate ABTS (2,2'-Azino-di(3-ethylbenzthiazoline) sulphonic acid; Zymed), the absorbance was read at 410 nm to obtain the concentration of sICAM-1.

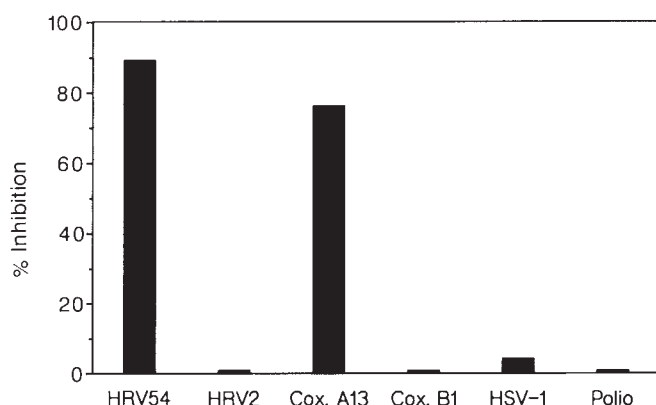


FIG. 3 Purified sICAM-1 specifically inhibits CPE induced by picornaviruses that use the major-group rhinovirus receptor. Purified sICAM-1 ($5 \mu\text{g ml}^{-1}$) was plated on HeLa cells with the viruses indicated (100 TCID_{50}), and cytopathic effect determined after 4 days: HRV54, major-group rhinovirus; HRV2, minor group rhinovirus; Cox. A13, coxsackie A13, a picornavirus using a major-group receptor; Cox. B1, coxsackie B1, which does not use a major-group receptor; Polio, poliovirus I; HSV-1, herpes simplex virus type-1. Data are representative of three experiments.

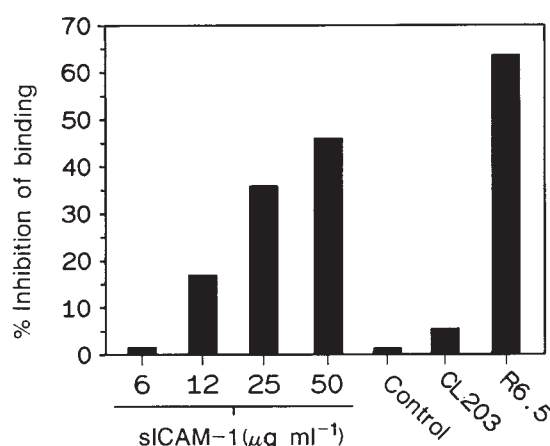


FIG. 4 Purified sICAM-1 inhibits the binding of rhinovirus virions to cells. [^{35}S]methionine-labelled HRV14 was mixed with sICAM-1, a chromatography-buffer control, or the anti-ICAM-1 monoclonal antibodies CL203 or R6.5 at $200 \mu\text{g ml}^{-1}$. As previously shown, antibody R6.5 inhibits the interaction of ICAM-1 with either LFA-1 or major-group HRV, whereas antibody CL203 does not³. After preincubation for 30 min at 4°C , the mixture was plated on HeLa cells, washed and counted for ^{35}S . Data are representative of two experiments.

METHODS. Radiolabelled rhinovirus binding was assayed using a modified method from ref. 20. Briefly, HeLa cells were infected with HRV14 for 4–6 h in methionine-free RPMI 1640 supplemented with 20 mM MgCl_2 and 2 mM glutamine, followed by incubation in the same medium with 2% fetal calf serum and $100 \mu\text{Ci ml}^{-1}$ [^{35}S]methionine until a generalized cytopathic effect was observed (usually 18 h post-infection). After 3 cycles of freezing and thawing, virus in the supernate was precipitated with polyethylene glycol. Virus was then pelleted through a 30% sucrose step gradient (34,900 r.p.m. for 2 h in a Beckman SW41 rotor). Binding of radiolabelled virus (1×10^4 c.p.m.) to HeLa cells (confluent 24-well plates) was as described²⁰, except that cells were solubilized by sequential washes with 1% Triton X-100 and hot 9 M urea before scintillation counting. The total volume of virus and antibody was 90 μl ; typically, 25–30% of $\sim 10,000$ input c.p.m. bound to cells.

was compensatory binding to an alternative receptor. Thus, a drug directed at the ICAM-1–virus interaction would have the dual advantages of intervening at the sensitive first stage of virus infection, combined with restricting any escape from neutralization through the generation of drug-resistant variants. □

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Transactivation by the hepatitis B virus X protein depends on AP-2 and other transcription factors

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THE hepatitis B virus (HBV) X gene product (pX) could be important in disease pathogenesis because it is known to transactivate transcription from many viral and cellular gene promoters^{1–8}, including the HBV core gene promoter, the human immunodeficiency virus (HIV-1) long terminal repeat, and the c-myc promoter. We have previously shown that only a subset of the promoters that can be transactivated by pX is transactivated in any particular cell line, and have proposed that pX acts through multiple, cell type-specific transcription factors⁹. We show here that pX acts through both AP-1 and AP-2 sites, and that pX has a transcription activation domain. We conclude that transactivation by pX depends on at least two distinct cellular DNA-binding transcription factors and we present a model for the action of pX.

We were previously unable to localize the *cis*-acting DNA sequences in the HIV-1 long terminal repeat that respond to transactivation by pX in Jurkat cells⁴. We have therefore turned to the SV40 enhancer, which has been intensively studied for its interactions with cellular transcription factors (see refs 10–14 for example), and is also transactivated by pX^{2,3,5}. The enhancer region of SV40 DNA was inserted into the reporter plasmid pMCAT3 to construct pA0MCAT (Fig. 1a). As expected, the presence of an upstream SV40 enhancer caused more than 10-fold transactivation of the promoter by pX in CV-1 monkey kidney cells (Fig. 1a). A series of plasmids containing SV40 enhancers with different clustered point mutations was constructed and similarly tested. Mutations in either the AP-1/downstream octamer or AP-2/NF- κ B binding sites (pA29MCAT and pA15MCAT, respectively) significantly reduced the level of transactivation by pX (Fig. 1a). A construct with mutations in both sites (pA1529MCAT) showed no significant transactivation by pX. By contrast, mutations in either the AP-3/NF- κ B or upstream octamer binding sites (pA12MCAT and pA23MCAT,

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respectively) did not interfere with transactivation by pX. Together these data indicate that pX acts through the AP-1 and AP-2 sites.

To confirm this inference, we tested whether pX could transactivate the pMCAT3 reporter plasmid, with multimeric AP-1 or AP-2 sites inserted upstream. Both constructs ([AP1]₄MCAT3 and [AP2]₅MCAT3, respectively) were transactivated by pX (Fig. 1b). This effect is sequence-specific, as the same promoter either with AP-3 sites ([AP3]₅MCAT3) or with mutant AP-2 sites, which bind poorly to AP-2 ([MAP2]₆MCAT3), was transactivated only ~3 fold (Fig. 1b).

In CV-1 cells, therefore, pX seems to act through cellular transcription factors AP-1 and AP-2. So-called AP-1 comprises the *fos* and *jun*-related families of proteins encoded by at least five genes (reviewed in ref. 14). Only one gene coding for AP-2 has been described^{15,16} and it is expressed in CV-1 cells (P.J.M., unpublished observations). To determine whether this is the transcription factor that mediates the effect of pX on AP-2 sites, we used *Drosophila melanogaster* Schneider-2 cells, which are devoid of AP-2 activity^{16,17}. The human AP-2 complementary DNA and HBV X gene were each included in *Drosophila* expression vectors driven by the ADH-2 promoter, and intro-

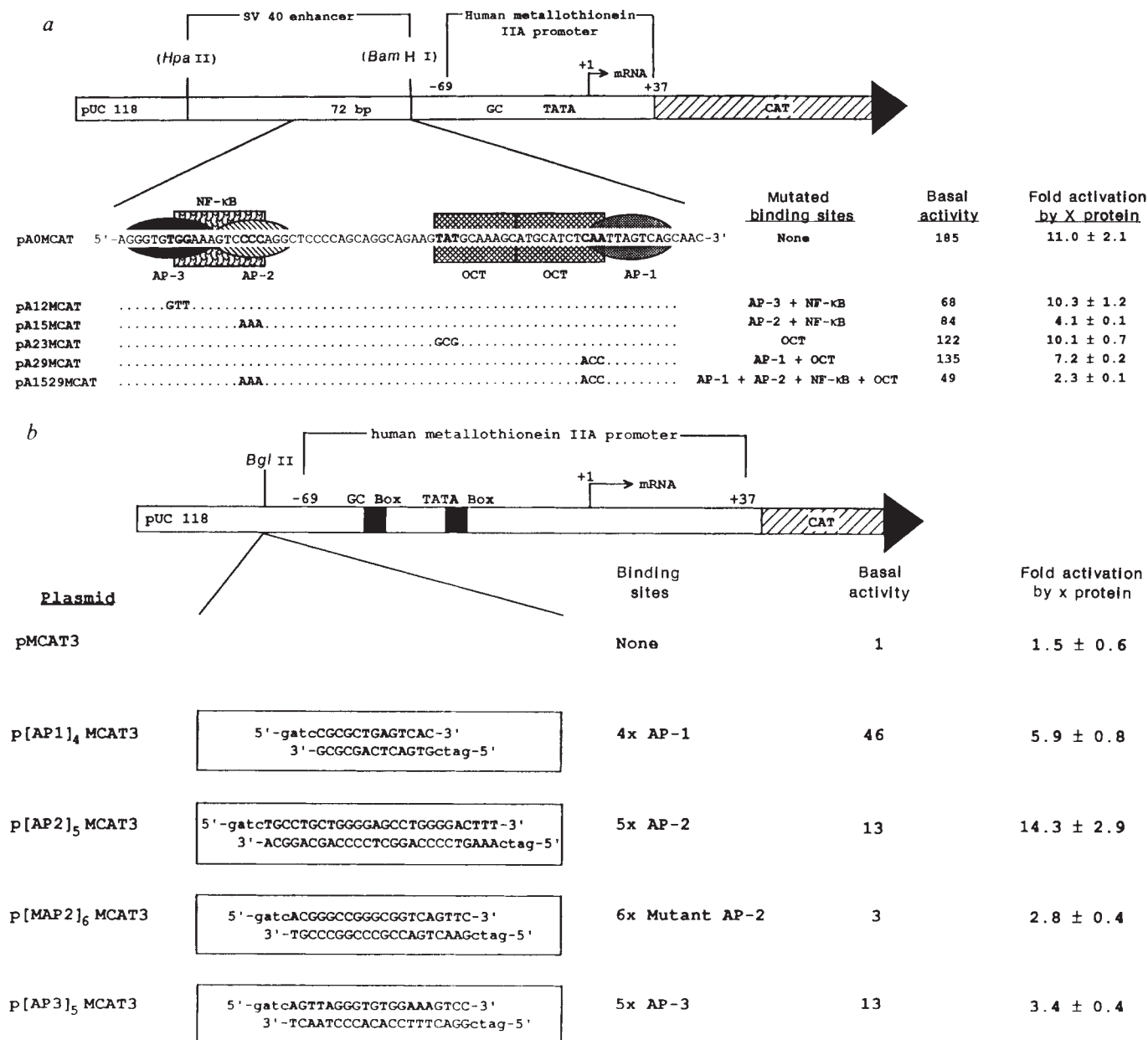


FIG. 1 Transactivation by pX *a*, of wild-type and mutant SV40 enhancers and *b*, of AP-1 or AP-2 sites. *a*, The indicated reporter plasmids were co-transfected into CV-1 cells with pECE-X, which expresses pX, or with pECE, the parental plasmid vector. After 2 days, the cells were collected and the CAT activity quantitated. Shown are the relative basal activities (normalized to pMCAT3), as well as the fold transactivation. The location of transcription factor binding sites on the SV40 enhancer is from refs 10-15. *b*, Similar experiments on derivatives of pMCAT3 with the indicated oligonucleotides inserted. The 'GC box' binds Sp1 (ref. 18). OCT, Octamer. METHODS. *a*, A 160-base pair fragment of the SV40 genome containing a single copy of the 72-bp enhancer and the upstream A domain, with or

without the indicated clustered mutations (derived from the pA0 series of plasmids¹⁰), was inserted upstream of a minimal human metallothionein IIA promoter in the CAT reporter plasmid pMCAT3 (ref. 24). The AP-1, AP-2, and AP-3 sites are from the SV40 enhancer¹³, but in reverse orientation relative to the early promoter; the mutated AP-2 site is derived from the metallothionein IIA promoter²⁵. Cells were transfected using the DEAE-dextran method⁹. The fold transactivation was calculated as the ratio of CAT activity in the presence of pECE-X to the activity in the presence of pECE. All numbers represent the mean (±s.d.) of 3 or more independent transfections.

TABLE 1 Transactivation of AP-2 sites in *Drosophila* cells

Protein expressed	Relative CAT activity
None	1.0
pX	0.6 ± 0.1
AP-2	5.5 ± 0.8
pX + AP-2	16.8 ± 3.4
Sp1	24.6 ± 3.9
pX + Sp1	9.0 ± 1.5

Human AP-2 cDNA¹⁶, Sp1 cDNA¹⁸ or pX (*NcoI/BglII* fragment of HBV strain adw²²) was inserted into the *Drosophila* expression vector pADH¹⁶. Schneider-2 cells were transfected by the calcium phosphate co-precipitation method²³, with 5 µg plasmid expressing each of the indicated proteins, 5 µg CAT reporter plasmid with Sp1 binding sites and five upstream AP-2 sites (p[AP2]₅MCAT; Fig. 1b), and, where necessary, enough pADH to make up a total of 15 µg.

TABLE 2 Transactivation by lexA-pX fusion protein

Protein expressed	Relative CAT activity		
	HIV-1	Lex(1)	Lex(2)
None (p6R control)	1.0	1.0	1.0
pX	11.8 ± 0.4	1.1 ± 0.3	1.7 ± 0.4
Truncated lexA	Not done	0.8 ± 0.2	0.9 ± 0.2
LexA-pX	13.1 ± 3.0	4.5 ± 0.3	5.7 ± 0.3

CV-1 cells were co-transfected with Rous sarcoma virus long terminal repeat-driven expression plasmid (derivatives of p6R²¹) and a CAT reporter plasmid driven by the indicated promoter construct (HIV-1, lex(1) or lex(2)). After 2 days, CAT activity was measured in cell lysates, and normalized to the activity in the p6R-transfected control lysates. The plasmid with the truncated lexA (plexΔZ) has been described²¹; the lexA-pX fusion construct was made by replacing the *XhoI/BglII* fragment of plexΔZ with the *NcoI/BglII* fragment of HBV²², together with a *XhoI/NcoI* linker. The HIV-1 CAT construct has been described⁴; the lex(1) and lex(2) plasmids were derived from pMCAT3 (Fig. 1) by insertion into its *BglII* site of the following *lexA* operator oligonucleotides: 5'CTCGAGTACTGTATGTACATACAGTACTCGA and 5'GATCGAATTCATTTTACTGTATATAAACACATGTGAATATACAGTTTTTGGTGTG, respectively.

duced separately or together in transient co-transfection assays with the pMCAT3 reporter plasmid containing upstream AP-2 sites. As expected, expression of AP-2 increases the chloramphenicol acetyltransferase (CAT) activity ~5 fold¹⁶, whereas pX alone has no significant effect (Table 1). When both pX and AP-2 are expressed, the CAT activity is almost 17 times base-line activity. The ADH-2 promoter of the *Drosophila* expression plasmid is not significantly transactivated by pX in Schneider cells (data not shown), ruling out the trivial explanation that pX increases the amount of AP-2 transcribed in the transfected cells. This effect is specific to AP-2 as pX actually decreases the activation of the same promoter by an unrelated transcription factor, Sp1 (ref. 18) (Table 1), possibly as a result of 'squenching'¹⁹. Therefore, human AP-2 can specifically mediate the transactivation effect of pX, even in a non-mammalian cell. These experiments also rule out the possibility that pX acts by directly binding to AP-2 sites. Similar experiments could not be performed with the plasmid containing AP-1 sites because this construct was transactivated by pX alone in Schneider cells (data not shown), a finding that is consonant with the fact that *Drosophila* cells contain homologues of mammalian AP-1 (ref. 17).

On the basis of these data, we suggest a possible model for the mechanism of pX action, namely that pX has a transcriptional activation domain, which is brought to the vicinity of a transcriptional initiation complex by the ability of pX to bind to multiple cellular factors, including AP-1 and AP-2. A similar model has been proposed for transactivation by the adenovirus E1A protein, and supported by data using fusion proteins with heterologous DNA-binding proteins²⁰. We therefore constructed

a fusion protein between pX and the DNA-binding domain of the bacterial repressor lexA²¹, thereby endowing pX with the ability to bind *lexA* operators. The fusion protein transactivates the HIV-1 long terminal repeat to roughly the same extent as native pX, but unlike pX, the fusion protein can also transactivate a minimal promoter with either of two different *lexA* operators inserted upstream (Table 2). A truncated *lexA* protein with the DNA-binding domain alone does not transactivate these constructs (Table 2). These findings are consistent with our model of pX action, and further experiments to confirm and refine our model are in progress. □

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Cell cycle oscillation of phosphatase inhibitor-2 in rat fibroblasts coincident with p34^{cdc2} restriction

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ATTENTION has focused on the regulation of the eucaryotic cell division cycle since the protein kinase p34^{cdc2} was identified as a key enzyme in mitotic induction¹⁻³. The level of this kinase remains constant throughout the cell cycle but its activity alters, particularly before M phase (for a review, see ref. 4). Although the factors regulating *cdc2* activity are still unknown, there is increasing evidence that it is influenced by p34^{cdc2} dephosphorylation⁵⁻⁷. Protein phosphatase inhibitor-2 (I2) is a specific inhibitor of phosphatase type-1 (refs 8, 9), which with type-2A is one of the two principal Ser(P) and Thr(P) phosphatases (reviewed in ref. 10). Here we show that the level of I2, assayed by immuno-

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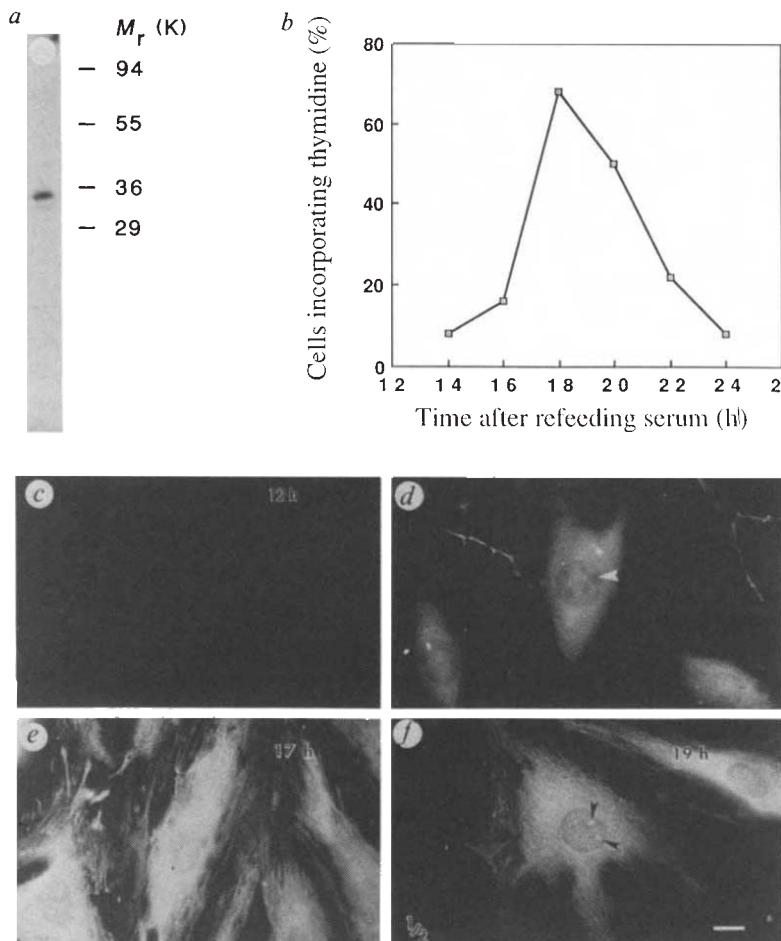


FIG. 1 Immunofluorescence staining for phosphatase inhibitor-2 (I2) in rat embryo fibroblasts during G1 and S phases. Synchronized REF-52 cells in G1 or S phase were stained for I2 distribution using monospecific affinity-purified immunoglobulins¹¹. The anti-I2 immunoglobulin specificity is shown by immunoblotting REF-52 cell total protein (a) Migration of molecular weight standards is indicated on the right. Cell synchrony is shown by thymidine incorporation after serum refeeding (b). Immunofluorescence staining of I2 is shown for cells in G1 (c), and early (d), mid (e) and late S phase (f). Scale bar, 5 μ m.

METHODS. Subconfluent REF-52 cells were blotted from a one-dimensional gel of half a 100-mm dish, before reacting with affinity-purified anti-I2 sheep antibodies (5 μ g ml⁻¹) as described previously²⁰. The intensity corresponds to 10–15 ng rabbit skeletal muscle I2 run as standard on the same gel. To assess the time of DNA replication (S phase), quiescent REF-52 cells were refed with serum, pulse-labelled for 2-hour intervals with [³H]thymidine, and the incorporation of [³H]thymidine into the nucleus visualized by emulsion autoradiography. *b*, Per cent of nuclei incorporating ³H versus time after refeeding allows us to define G1 as 0 to 16 h (<20%), S phase as 16–22 h (>65–70%), and G2 from 22 h to mitosis (about 26–28 h) (<10%). For immunofluorescence, REF-52 cells grown on glass coverslips²¹ were quiesced by serum depletion for 36 h. G1 cells (c) were fixed 12 h after serum refeeding. Cells in early, mid or late S phase, were processed 16, 17 and 19 h after serum refeeding (d, e and f). Cells were fixed as described²¹ before staining for I2 with 10 μ g ml⁻¹ anti-I2 immunoglobulins¹¹, visualized by incubation with biotin-conjugated anti-sheep/goat and fluorescein-streptavidin. In c, d and e, exposure times were identical, but in f, exposure was 2-fold lower to facilitate identification of structural detail. We obtained identical staining results for I2 in S phase (corresponding to d and f) using 2 mM hydroxyurea to block cells before their entry into S phase.

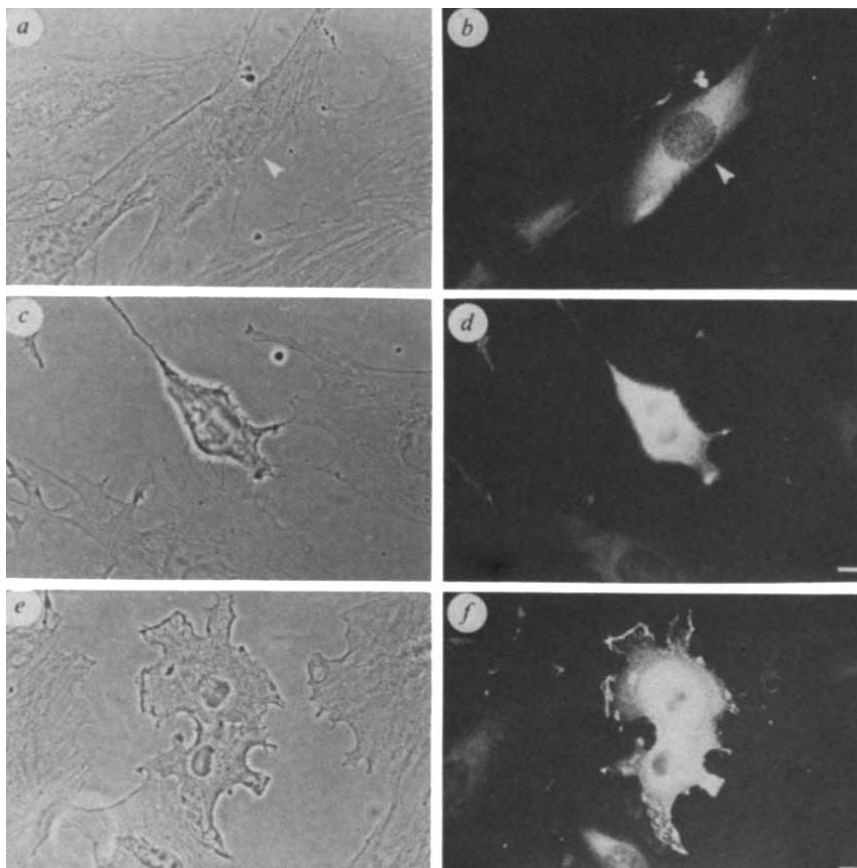


FIG. 2 Staining for phosphatase inhibitor-2 rises continuously during mitosis. REF-52 cells were synchronized by serum starvation and 27 h after refeeding, were fixed and stained with anti-I2 antibodies as described for Fig. 1. Phase (a, c, e) and fluorescent (b, d, f) micrographs of cells are shown at prophase (a, b), anaphase (c, d) and telophase (e, f). The images in b and d were exposed manually and printed in the same way, whereas that in f was printed at half the exposure time to avoid obliterating details in the telophase cell. The staining intensity of the telophase cell in f is at least 10-fold higher than the background cells, as assessed by densitometry. (Note the two cells have clearly defined nuclei in the phase image in e.) Separate negatives of the same frame including these cells were recorded on either Kodak technical pan 50 ASA or TriX pan 400 ASA. Arrows in a and b mark a prophase cell containing condensed chromatin.

fluorescence staining, activity measurements, western immunoblotting and metabolic labelling, oscillates during the cell cycle in rat fibroblasts, peaking at S phase and mitosis. Moreover, when we inhibited I2 *in vivo* by microinjection of anti-I2 antibodies in S-phase cells, the pseudo-mitotic cellular response to injected p34^{cdc2} was restored, indicating that I2 might have a role in the modulation of p34^{cdc2} activity.

Expression of I2 during the cell cycle of rat embryo fibroblast (REF-52) cells was initially assayed by immunofluorescence, using a polyclonal sheep antibody affinity-purified against rabbit skeletal muscle I2 (ref. 11). This antibody recognizes a single protein (relative molecular mass (M_r) 33,000 (33K)) which by immunoblotting corresponds to I2 in REF-52 cells (Fig. 1a). REF-52 cells were synchronized by serum deprivation for 36 h. After readdition of serum, the cells had a doubling time of 26–28 h. To improve the definition of different cell cycle phases, cells refed with serum were pulse-labelled for 2-hour intervals with [³H]thymidine. Figure 1b shows how the proportion of cells incorporating ³H was greatest at 18–19 h and that most incorporation occurred between 16 and 22 h. Under these condi-

tions we can distinguish three cell cycle phases: G1, 0–16 h; S, 16–22 h; G2, 22 h-mitosis.

During G1, cells show low-intensity immunofluorescent staining of the cytoplasm (Fig. 1c), at a level that is consistent in all cells up to 12 h. As cells progress from G1 into S phase, 15–16 h after refeeding, the intensity of immunofluorescent staining for I2 increases sharply in both the cytoplasm and nucleus (Fig. 1d). This increase in overall fluorescence is observed in 60–70% of the cells at that time, and by 17 and 19 h after serum readdition, all cells are brilliantly stained throughout the nucleus and cytoplasm (Fig. 1e, f). Similar results are obtained with cells re-entering S phase after release from hydroxyurea (data not shown). The level of staining in S-phase cells is 8-fold higher than in G1 cells, as estimated by scanning densitometry of the photographic negatives.

Progression of cells from S phase to G2 is accompanied by a marked diminution of immunofluorescent staining for I2 to a level comparable to that seen in G1 cells. This low level of I2 staining is found in at least 70% of the cells throughout the entire G2 period (see background cells in Fig. 2). As cells enter

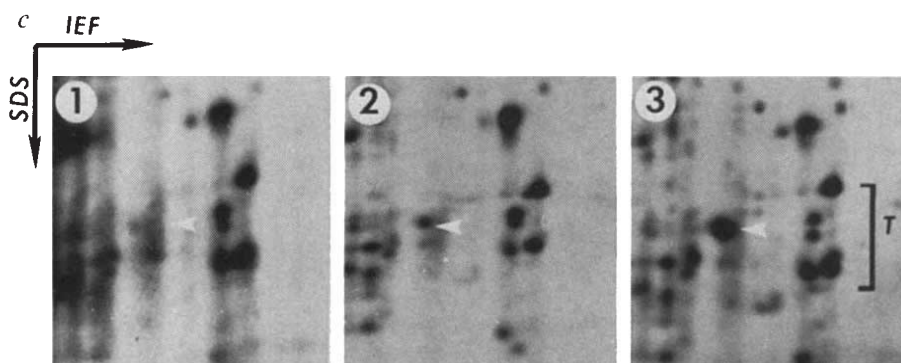
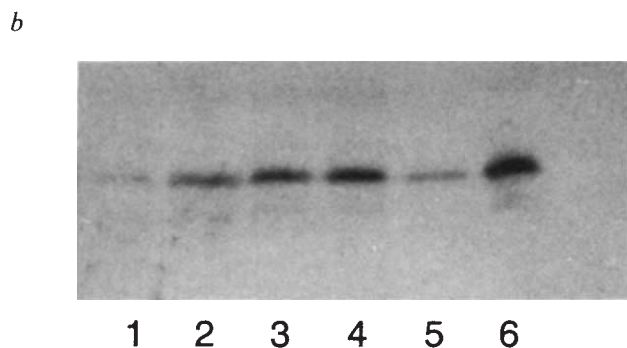
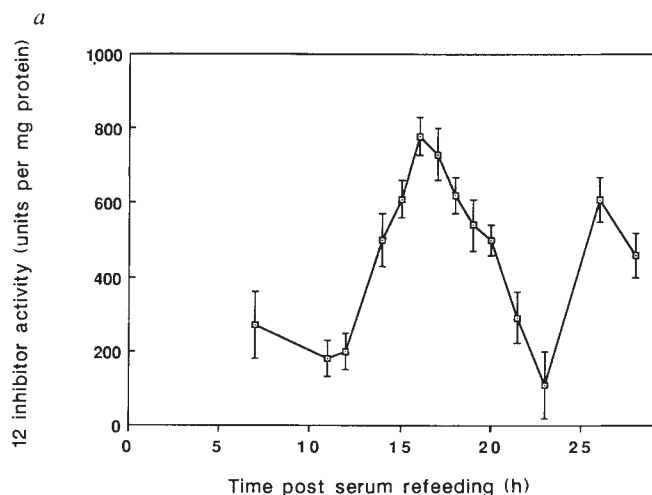


FIG. 3 Oscillation of protein phosphatase inhibitor-2 during the cell division cycle. Changes in the abundance of I2 during the cell cycle were confirmed in synchronized REF-52 cells by analysing inhibitory activity against rabbit skeletal muscle phosphorylase (type-1) phosphatase (a), anti-I2 immunoblotting (b) and [³⁵S]methionine pulse-labelling (c).

METHODS. Subconfluent REF-52 cells were synchronized by serum starvation. At different times after serum readdition, cells on 100-mm plates were lysed in 400 μ l lysis buffer (20 mM MOPS, pH 7.4, 0.1% Triton X-100) before heating to 100 °C for 5 min. After clarification by centrifugation, supernatants were assayed for I2 activity as described¹⁴. The average values for triplicate determinations were normalized for heat-stable protein content (0.18 to 0.26 mg ml⁻¹). The pattern of change in inhibitory activity over time (low in G1, 6–12 h; high in S phase, 16–20 h; low in G2, 21–23 h; and high at mitosis, 26–28 h) was replicated in several separate experiments using different stocks of REF-52 cells and the profile shown is a compilation of 4 different experiments, showing the maximum deviation for each point. For immunoblotting, cells from 100-mm plates were processed as described in Fig. 1a, with lanes 1–6 (left to right) corresponding to $t=12, 16, 17.5, 20, 23$ and 26 h after serum readdition. The apparent difference between the high levels of I2 on the blot at mitosis (lane 6) and the corresponding relatively decreased activity (lane 1) results from the doubling of cells at M phase, which increases the level of I2 on the blot while its activity (expressed as units per mg) is diminished by the increased amount of proteins. Synthesis of I2 during the cell cycle was analysed by pulse-labelling with REF-52 cells synchronized by serum starvation (G0) and after refeeding serum for 12 h (G1) or 16 h (S phase). 60-mm dishes and cells were labelled for 90 min with 200 μ Ci [³⁵S]methionine and labelled proteins analysed by two-dimensional gel electrophoresis and fluorography using Amplify (Amersham). The location of I2 (indicated by arrowheads) was determined by western immunoblotting of a duplicate sample of S phase cells. The region of the autoradiograph from the gels containing I2 is shown with acidic side to the right and with M_r decreasing from top to bottom.

prophase (Fig. 2*a, b*), the level of I2 staining rises in the cytoplasm, when these cells appear much brighter than the surrounding G2 cells (note that the lower left-hand cell is also entering mitosis). As division progresses, I2 staining continues to increase through anaphase (Fig. 2*c, d*) and telophase (Fig. 2*e, f*). The level of I2 staining peaks in flattening telophase cells (Fig. 2*e, f*), which implies that increased I2 staining does not simply reflect the rounding and compaction of cells during mitosis. Moreover, scanning densitometry reveals a grain density (for Fig. 2*f*) in the telophase cell that is 10-fold higher than that in the surrounding G2 cells. As cells complete telophase, I2 staining decreases, so that within 1 hour after mitosis it is comparable to the level in G1 cells (Fig. 1*a*).

We verified that I2 immunofluorescent staining fluctuated during the cell cycle, reaching a peak during S phase and mitosis, by measuring the activity of a heat-stable and trypsin-sensitive phosphatase-1 inhibitor¹¹. Figure 3*a* shows that increased I2 activity coincides both with S phase (16–20 h) and mitosis (26 h), but decreases during G1 and G2 (6–12 h and 21–23 h, respectively). These observations were confirmed by western immunoblots: I2 levels during the cell cycle (at 12, 16, 17.5, 20, 23 and 26 h) are shown in Fig. 3*b*. Again, the amount of I2 peaks during S phase and at mitosis, subsiding to low levels during G1 and G2. We have also shown that I2 levels and activity increase at mitosis using cells blocked at metaphase by nocodazole (data not shown).

Enhanced synthesis of I2 during S phase is also evident when synchronized REF-52 cells are pulse-labelled with [³⁵S]methionine. We labelled synchronized cells with 200 μ Ci [³⁵S]methionine for 90 min, in either quiescent (G0) cells or 12-h (G1) and 16-h (S-phase) cells after refeeding serum. Labelled proteins were analysed by two-dimensional gel electrophoresis and autoradiographs of the relevant areas of the gels are shown in Fig. 3*c*. The position of I2 was determined by western immuno-

blotting of a duplicate two-dimensional gel with anti-I2 immunoglobulins (data not shown). Synthesis of I2 proceeds at a low level during G0 (Fig. 3*c*, 1) and G1 (Fig. 3*c*, 2), but increases sharply as cells enter S phase (Fig. 3*c*, 3). I2 synthesis can be gauged by comparing its labelling with the five tropomyosin proteins that are produced in large amounts throughout the cell cycle¹². Our findings show that the high levels of I2 detected at S phase by immunofluorescence (Fig. 1), activity measurements (Fig. 3*a*) and western immunoblotting (Fig. 3*b*) result from an increase in the synthesis of I2 during S phase, demonstrating that I2 is a new cycling protein. Indeed, degradation of I2 during the cell division cycle is not unexpected, considering its extreme sensitivity to proteases *in vitro* (complete degradation in <1 min by trypsin, with no fragments detectable by western blotting) and the fact that ~40% of the protein is composed of the 'PEST' sequences¹³ characteristic of proteins having a rapid turnover, such as *myc* and *fos* proteins and E1A, as well as I2 (ref. 14).

This apparent cell cycle modulation of I2 prompted us to investigate whether it was involved in the regulation of the activity of the ubiquitous p34^{cdc2} kinase. We have recently shown that microinjection of p34^{cdc2} into G0 or G1 REF-52 cells rapidly produces extensive changes in cell shape, cytoskeletal organization and chromatin structure¹⁵. Interestingly, microinjection of cells during S phase has no effect, and the drastic effects seen during G1 are reversed when the injected cells enter S phase¹⁵. Because p34^{cdc2} kinase activity seems to be attenuated at S phase, a time that coincides with an increase in I2, we tested whether co-injection of anti-I2 immunoglobulins with p34^{cdc2} kinase could restore the response of cells to the kinase at S phase. The results of microinjecting p34^{cdc2} kinase or anti-I2 immunoglobulins alone, or the two preparations together into synchronized REF-52 cells in S phase are shown in Fig. 4. Figure 4*A* is the phase image of cells injected with p34^{cdc2} alone, which

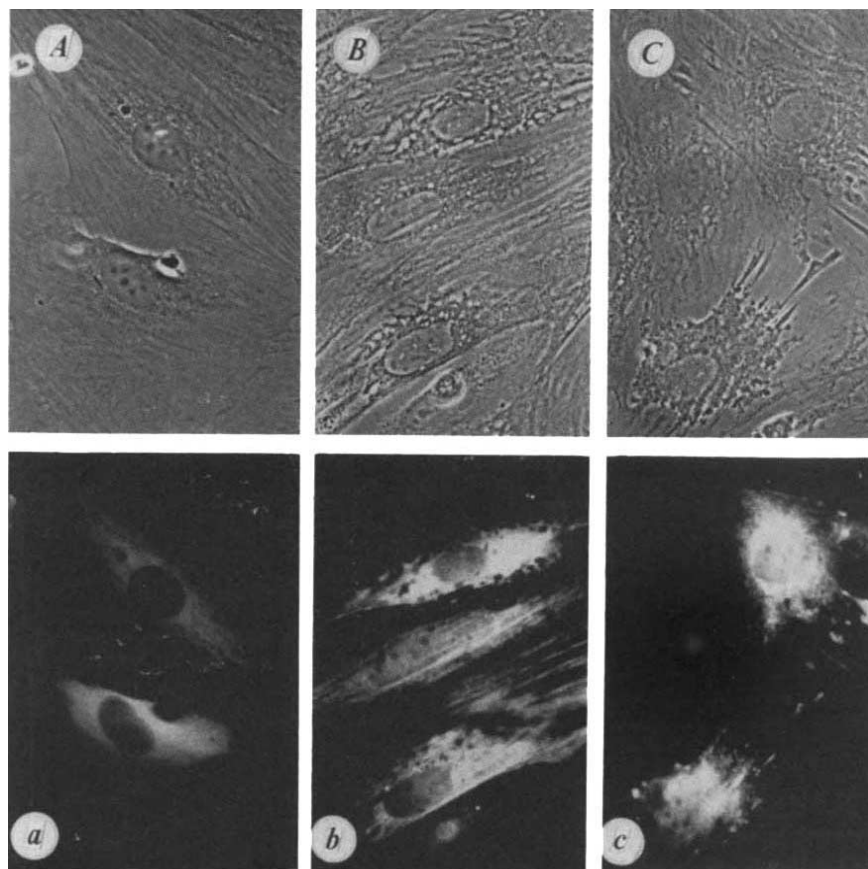


FIG. 4 Microinjection of anti-I2 antibodies restores the *in vivo* activity of starfish p34^{cdc2} kinase in REF-52 cells during S phase. Phase (A, B and C) and fluorescence (a, b, c) micrographs of synchronized REF-52 cells are shown at S phase after coinjection with 0.75 mg ml⁻¹ activated p34^{cdc2} kinase (A, a), 1.5 mg ml⁻¹ anti-I2 antibodies (B, b) or a solution of p34^{cdc2} kinase plus anti-I2 antibodies (C, c). The injection buffer contained an inert non-specific goat antibody as a tracer to allow identification of injected cells, visualized after fixation by staining with a fluorescent secondary antibody (a, b and c).

METHODS. REF-52 cells were synchronized at S phase using 2 mM hydroxyurea. Similar results were obtained with cells synchronized by addition of serum to quiescent cells. Kinase and anti I2 immunoglobulins were dialysed against injection buffer²¹, then injected into cells 60 min after removal of the hydroxyurea block. Cells were fixed 60 min after injection in 3.7% formalin, extracted with 0.1% Triton X-100, and reacted with biotin-conjugated streptavidin to visualize the injected cells by staining of the tracer antibody (shown in a, b and c). The anti-I2 immunoglobulin were raised in sheep and also stained in this procedure.

clearly shows that there is no overall change in cell morphology, confirming our previous observation¹⁵; cells injected with anti-I2 immunoglobulins alone (3 cells stained in Fig. 4b) do not show any shape change either (Fig. 4B and b). But when the cells are injected with both together, there is a characteristic change in cell shape (Fig. 4C and c): the injected cells (65 out of 70) round up and lose cell-surface contact, a response similar to that after microinjection of p34^{cdc2} in G0 or G1 (ref. 15). These results show that co-injection at S phase of antibodies that neutralize I2 can restore the morphological effects induced by p34^{cdc2} in REF-52 cells.

The fact that p34^{cdc2} protein kinase is itself present throughout the cell cycle¹⁶ calls for a regulatory mechanism involving other oscillatory protein(s). Our findings support the idea that variations in I2 concentration could modulate p34^{cdc2} activity, but we cannot yet say whether this reflects a direct binding of I2 to p34^{cdc2} kinase or to its associated subunits (for example, cyclin), or is an indirect effect by I2 on the phosphorylation status of kinase subunits. The wide phylogenetic conservation of I2 in *Drosophila*^{17,18}, invertebrates¹⁹ and mammals, is consistent with our notion that this protein is a component of an essential regulatory circuit. Our observation of I2 oscillation during the cell division cycle indicates that there are major changes in the abundance of this protein coincident with two critical periods,

namely DNA synthesis and mitosis, and provides evidence that I2 is specifically involved in the modulation of cell cycle dynamics. □

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Are cross-regulatory interactions between homoeotic genes functionally significant?

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THE first instar larva of *Drosophila* consists of a chain of segments or parasegments¹ in which the morphological pattern characteristic of each metamere is determined by the homoeotic genes^{2–4}, which are active in overlapping domains and are known to interact among themselves^{5–9}. The interactions occur at the level of transcription and allow some homoeotic genes to control the patterns and levels of expression of others. The best known among them are the down-regulation of *Antennapedia* (*Antp*) by *Ultrabithorax* (*Ubx*) and that of *Ubx* by *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*). It has been proposed^{7,10,11} that these cross-regulatory interactions play a part in specifying cell pattern, and hence the identity of each metamere. Here we assess the functional significance of some of these interactions by expressing the *Antp*, *Ubx* or both homoeotic genes under the control of the heat-shock promoter^{12–14}. Predictably, we find that homoeotic gene products evade normal regulatory controls and can be maximally expressed in regions where they are normally down-regulated, but, surprisingly, we find that interruption of the normal down-regulation of *Antp* and *Ubx* has no phenotypic consequences in the epidermis, where homoeotic phenotypes are normally manifest. Hence our results challenge the view that these, and possibly other cross-regulatory interactions have a role in determining segmental identity.

Embryos carrying *hsp70-Antp* or *hsp70-Ubx* fusion genes were heat-shocked during the extended germ band stage (~5–6 h old) to induce high levels of *Antp* or *Ubx* protein in all cells of the embryo; the consequences to development were then analysed by assaying the cuticular patterns of the larval segments. In some experiments, embryos carrying both fusion genes were

heat-shocked to induce simultaneous expression of both proteins.

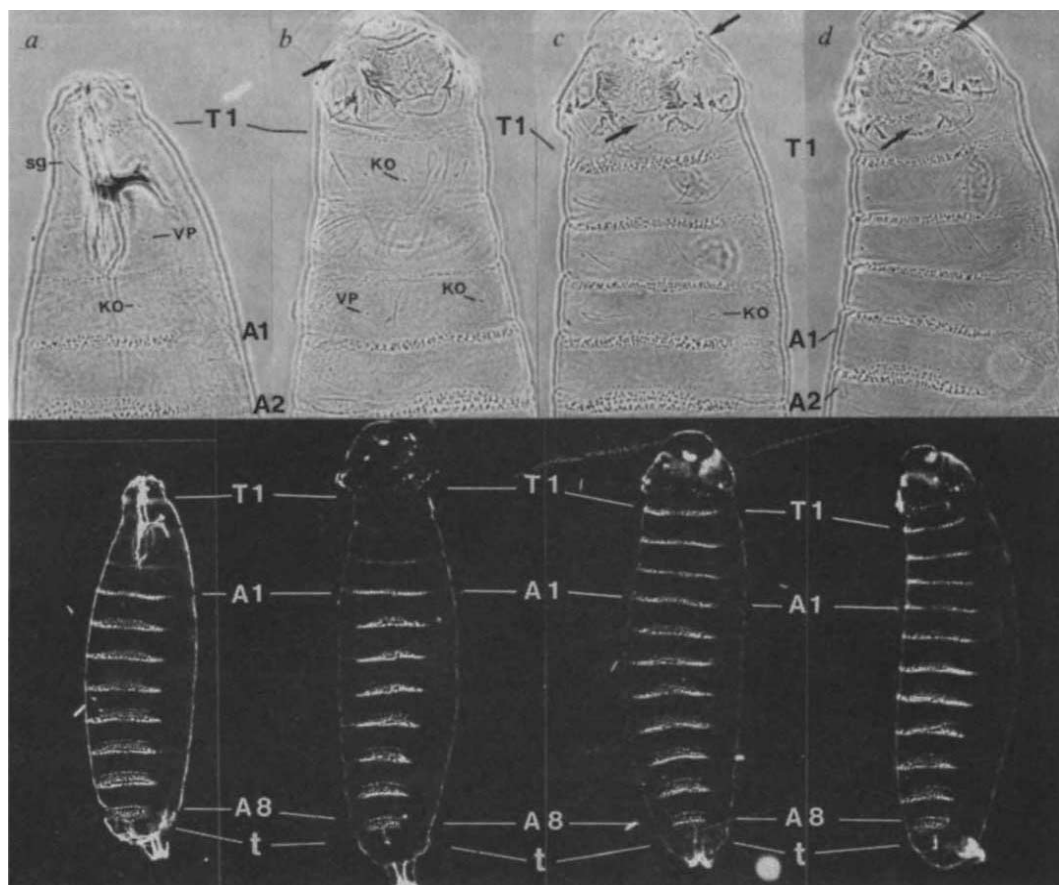
To induce ubiquitous expression of *Antp*, we used flies of H4 stock¹²; these flies carry the *hsp70-Antp* fusion gene (*HSA*) inserted in the third chromosome, so the homozygous stock carries two *HSA* elements. After heat shock, all cells express high levels of *Antp* protein for several hours (see legends to Figs 1 and 2, and ref. 13). This expression of *Antp* causes a profound alteration of head and prothoracic (T1) segments, in which many of the distinctive pattern elements are replaced in part by mesothoracic (T2) ones. But T2 itself, the more posterior segments and the telson are all normal (Fig. 1). The modification of the cephalic and T1 segments is further evidence that *Antp* overrules or suppresses the genes determining head involution and identity^{13,15,16}. Likely candidates are *labial*^{17,18}, *Deformed*^{19,20} and *Sex combs reduced*²¹, although overexpression of *Antp* in T1 and cephalic segments does not significantly suppress the expression of *Scr* protein¹³. By contrast, the lack of effect in the region from T2 to the telson indicates that the down-regulation of *Antp* by *Ubx* and *abd-A* that normally occurs in most of the region^{6,9,10,22,23} can be overridden without any change in segmental morphology. We emphasize that *Antp* protein induced from the *HSA* gene cannot be down-regulated.

For ubiquitous expression of *Ubx*, we used an *hsp70-Ubx* construct (*HSU*) containing a *Ubx* coding sequence from class Ia *Ubx* complementary DNA²⁴. (See the legend to Fig. 1 for details of the construct and of the treatment.) This is expressed in the epidermis. The stock bears *HSU* in the third chromosome and is homozygous.

After a standard heat-stock treatment, expression of *Ubx* protein is uniformly strong and persistent (Fig. 2b). There is a profound effect on the identity of the cephalic and thoracic segments: all segments anterior to the first abdominal segment (A1) acquire partial or complete A1 character, which during normal development is associated with high uniform expression of *Ubx*. The body region from A2 to the telson remains unaltered (Fig. 1).

The change of identity of cephalic and thoracic segments towards A1 indicates that the ectopic expression of *Ubx* overrules cephalic and thoracic genes, including *Antp*, which normally functions in the thoracic segments. By contrast, the absence of any effect in the abdominal segments posterior to A1 indicates that the normal down-regulation of *Ubx* by *abd-A*

FIG. 1. Ventral view of *a*, wild-type; *b*, HSA/+; *c*, HSU/+; and *d*, HSA/HSU larvae after heat shock. Lower panel (dark field), entire segment patterns of larvae; upper panel, head, thoracic and the first two abdominal segments. Note that in the wild-type larva (*a*) the T1 segment occupies a most anterior position as a result of the invagination of the head structures that come to be situated beneath the thoracic cuticle. Segments T1, T2, A1, A2, A8 and telson (t) are indicated. In T1 the denticles are larger than those of T2 and T3 and there is also a second group (sg) of ventrally located denticles that do not appear in T2. The A1 segment is different from the thoracic segments in that the denticles are bigger and it does not contain structures like Keilin's organs (KO) or ventral pits (VP). The denticles of A2 are like those of A1 but they are arranged differently, forming a broader belt. HSA/+ larva (*b*) obtained after outcross of the HSA homozygous stock to wild type were heat shocked for 1 h when they were 5 h old. The larva contained one dose of the *hsp70-Antp* fusion gene¹². Note that only segments anterior to T2 are modified by the heat shock. The body region from T2 to the telson shows a normal pattern. In the abdominal segments, for example, no thoracic markers like KO or VP are ever seen. T1 is partially transformed towards T2, as indicated by the arrow in the head, indicating that the T1 segment of the larvae (*c*) were obtained after transformation of the wild-type. The *hsp70-Ubx* fusion gene was transformed into the wild-type sequence was assembled from upstream of the translation start site; (2) a ~215 bp *BsmI-XbaI* fragment which spans the principal 5' microexons; and (3) a 3' *XhoI*-*NotI* fragment which spans the position ~560 bp downstream of the start site. The fusion gene was then inserted into a C20 vector and 3' transcription terminating. The effects of the heat shock on the development of the larva are involution is prevented, as in HSA transformation towards A1, indicating that the thoracic landmarks. Note the presence of the thoracic markers in the HSA/HSU larvae (*d*) were obtained after outcross of the HSA/HSU stock to wild type.



Inter se. The set of transformations in this larva are like those of HSU⁺, both in the head (inhibition of involution, presence of abdominal denticles) and in the thoracic segments, transformed towards A1. No effect is seen posterior to the A2 segment, in spite of strong *Antp* and *Ubx* overexpression.

METHODS. For precise timing in heat shock treatments, dechorinated embryos were selected at stage 6 (2 h 50'–3 h), when the cephalic furrow becomes visible and the pole cells begin to migrate. Different batches of embryos were then allowed to develop for 0, 1, 2, 3, 4, 6 or 9 h before being transferred to a 36 °C bath for 1 h, thus ensuring at least 4 h of heat-induced protein (see legend to Fig. 2). Microscopic examination after completion of the embryonic period indicated that morphological effects were most pronounced in embryos 5 or 6 h old, so embryos of this age were used to investigate the effects of heat shock on different body parts. Heat shock had no effect in embryos older than 9 h, even though heat-induced protein persists for several hours. For HSA/+ and HSU/+, we sometimes used a multiple heat-shock treatment, giving three pulses of 20 min to the same embryos at 4, 6 and 8 h; for *Ubx* this induces a high level of protein through all the critical stages of embryogenesis up to 14–16 h of development. After this treatment the effect on the head is more pronounced (A.G.-R. and G.M., unpublished results) than after 1 h of heat shock, but there is no difference in the thorax and abdomen.

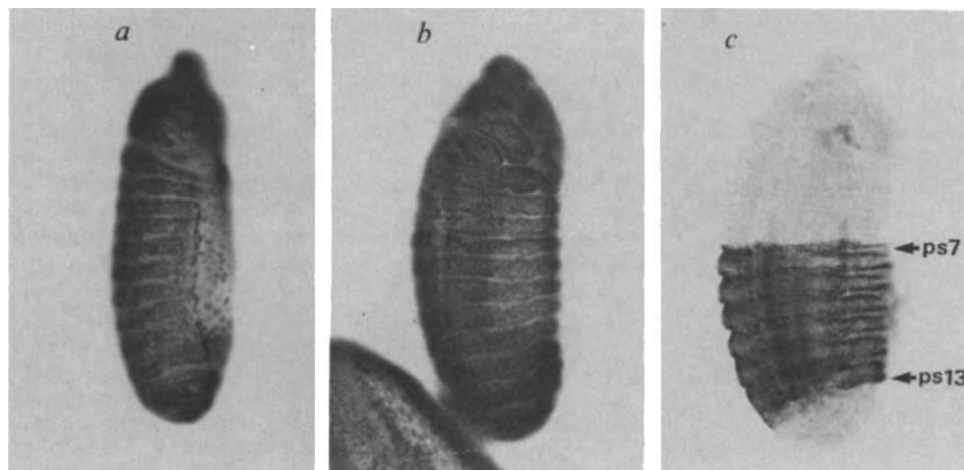


FIG. 2 Immunolocalization of *a*, *Antp*, *b*, *Ubx*; *c*, *Abd-A* proteins in heat-shocked HSA/HSU embryos. After heat shock for 1 h and recovery for 3 h, embryos were fixed for staining at ~10 h of age, after germ band retraction. High levels of *Antp* and *Ubx* proteins persist for 3 h after the end of the heat shock. Note that embryos in *a* and *b* are intensely labelled in all nuclei, indicating that all cells express both *Antp* and *Ubx*. The embryo in *c* expresses *abd-A* protein normally from parasegment 7 to 13. All embryos originate in the same cross and were stained using a standard Vectastain-ABC kit (Vector). Antibodies used were: monoclonal anti-*Antp* (from D. Brower) monoclonal anti-*Ubx* (from R. White), and an anti-*abd-A* polyclonal (from J. Casanova).

and *Abd-B* in the central nervous system and the epidermis in this region⁷ could be functionally irrelevant. This is particularly striking in parasegment 13 (~A8), where *Ubx* is severely repressed in wild-type embryos but is highly expressed in HSU embryos after heat shock. Hence, the influence of these genes that act more posteriorly overrides the effect of *Ubx* protein expression in these segments. In HSU embryos homozygous for *Df(3R)109* (lacking both the endogenous *Ubx* and *abd-A* genes), we observe that not only are the head and thoracic segments transformed towards the A1 segment type, but that the abdominal segments A1–A4, in which *Ubx* and *abd-A* are normally active, also show the same transformation. In addition, this experiment shows that the developmental effects of HSU are not produced by activation of its own endogenous gene, unlike the case of the *hsp70-Dfd*¹⁴.

These findings indicate that there could be a functional hierarchy between the abdominal, *Ubx* and *Antp* genes that is independent of their cross-regulatory interactions. Accordingly, even if down-regulation of *Antp* by *Ubx* is overridden by forced *Antp* expression from the *HSA* gene, normal function of *Ubx* in parasegment 5 and 6 (~T3, A1) still ensures that these regions develop normally. Similarly, the normal activity of *abd-A* and *Abd-B* still dictates wild-type segmental patterns in parasegments 7–13, even when high levels of *Ubx* expression cannot be prevented.

We investigated this possibility further using embryos containing *HSA* and HSU, in which heat shock produces simultaneous and irrepressible expression of both *Antp* and *Ubx* gene products and expression of the *abd-A* protein is normal (Fig. 2). We find that heat-shocked HSA/HSU embryos give rise to larvae indistinguishable from those derived from heat-shocked HSU/+ embryos (Fig. 1). Hence, high-level *Antp* expression seems to be inconsequential when *Ubx* is comparably expressed. Moreover, the simultaneous expression of both *Antp* and *Ubx* has no effect on abdominal patterning because of the normal expression and function of the *abd-A* (Fig. 2) and *Abd-B* genes.

In homoeotic gene function it is considered that the functional hierarchy among homoeotic genes^{13,15,16} is based on cross-regulatory interactions that modulate the level appropriate for each metamere^{11,22,24–26}. But our results argue that regulation between *Antp*, *Ubx*, *abd-A* and *Abd-B* is functionally irrelevant, at least in the epidermal cells giving rise to the larval cuticle. Hence they question the significance of other cross-regulatory interactions observed between homoeotic genes, particularly those seen in the central nervous system and the somatic and visceral mesoderm^{6–9,23}. Our results also question how this functional hierarchy arises between homoeotic genes^{5,13,15,16}. There could be competition for 'downstream' subordinate genes¹³. The homoeotic genes presumably control large overlapping sets of subordinate target genes. Because the homoeodomain characteristic for homoeotic proteins is a sequence-specific DNA-binding domain²⁷ and proteins containing a homoeobox are transcriptional regulators, the homoeotic proteins are thought to bind to regulatory sequences in these target genes. The differences between *Antp* and *Ubx* within the homoeodomain and in the flanking sequences are enough to account for any difference in affinity for the same or similar binding sites in the subordinate genes. Competition and differential affinity for similar binding sites or competition for other transcription factors could account for the hierarchy of effects that we find, but their molecular basis remains to be elucidated. □

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A 3' splice site-binding sequence in the catalytic core of a group I intron

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RIBOZYMES use specific RNA–RNA interactions for substrate binding and active-site formation. Self-splicing group I introns have ~70 nucleotides constituting the core, a region containing sequences and structures indispensable for catalytic function^{1–3}. The catalytic core must interact with the substrates used for the two steps of the self-splicing reaction, that is, guanosine, the 5'-splice-site helix (P1) and the 3' splice site. Mutational evidence suggests that core sequences near segment J6/7 that joins the base-paired stems P6 and P7, and the bulged base of P7(5'), participate in binding guanosine substrate^{4–5}, but nothing is known about the interactions between the core, the 5'-splice-site helix and the 3' splice site. On the basis of comparative sequence data, it has been suggested that two specific bases in the catalytic core of group I introns might form a binding sequence for the 3' splice site⁶. Here we present genetic evidence that such a binding site exists in the core of the *Tetrahymena* large subunit ribosomal RNA intron. We demonstrate that this pairing, termed P9.0, is functionally important in the exon ligation step of self-splicing, but is not itself responsible for 3'-splice-site selection.

The two bases immediately downstream of conserved sequence element *S* constitute the putative binding site for intron sequences at the 3' splice site (Fig. 1). These sequences lie between two short intramolecular RNA helices, termed P7 and P9, that are invariant features of the group I core secondary structure^{7–8}. It has been proposed that in *Tetrahymena* large subunit rRNA intron (*LSU*), bases G313 and A314 pair with C413 and U412, the two bases immediately upstream of the universal G at the extreme 3' end of the intron⁶. We tested this hypothesis by analysing the effect of compensatory mutations on splicing. According to the proposed model, either of the two-base mutations G313→U and A314→U or U412→A and C413→A would be expected to disrupt the putative P9.0 pairing, whereas the four-base mutation G313→U, A314→U, U412→A and C413→A would restore P9.0 (Fig. 1).

We constructed the mutants described above by oligonucleotide-directed mutagenesis using a phagemid construct containing *Tetrahymena LSU* cloned as a functional intron in the *Escherichia coli lacZα* coding sequence as previously described⁹.

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The core and 3'-splice-site mutations each gave rise to white colonies on media containing 5-bromo-4-chloro-3-indolyl- β -D-galactoside (X-gal), whereas the compensatory mutant and wild type gave rise to blue colonies. Therefore, mutations at the targeted sites inhibit and restore β -galactosidase expression in the hybrid gene construct, supporting the proposed pairing interaction.

We confirmed and extended these results by *in vitro* RNA processing experiments (Figs 2 and 3). We transcribed radio-labelled precursor RNAs from mutant and wild-type DNA templates, and analysed self-splicing activity. For the core and 3'-splice-site mutants, excision of intron sequences occurred at a rate equivalent to that of wild type, indicating that guanosine cleavage at the 5' splice site was unaffected and that cleavage of the 3' splice site could proceed in an efficient manner. But, there was a striking difference in the efficiency of exon ligation. As splicing of wild-type preRNAs proceeded, spliced exons accumulated in significant molar excess over unspliced exons. By contrast, the mutants at the 3' splice site and in the core showed a defect in exon ligation activity, with 50% or more of the exons being released in unligated form. These results indicate that a mutation in the catalytic core, at a site distant from the 3' splice site in the primary structure, can specifically inhibit the exon ligation step of the splicing reaction. In the P9.0[5'] and P9.0[3'] mutants, cleavage at the 3' splice site probably proceeds by hydrolysis, although a mechanism involving exon ligation followed by hydrolysis cannot be ruled out. The finding that intron sequences immediately upstream of the 3' splice site are important in exon ligation is consistent with previously published work¹⁰⁻¹¹. The splicing phenotypes of the core and 3'-splice-site mutants are nearly identical, the only significant difference being the appearance of a minor uncharacterized circular RNA species in the 3'-splice-site mutant (Fig. 3).

Analysis of the four-base mutant revealed that the base substitutions in the core and 3'-splice-site mutants act in a compensatory manner when incorporated into the same preRNA, restoring efficient exon ligation activity (Figs 2 and 3). No significant differences have been identified between the preRNA processing reactions of the compensatory mutant and wild-type preRNAs. This latter observation is consistent with the finding that the sequences involved are not conserved among group I introns^{3,6}.

In most cases, splicing defects caused by genetic disruption of essential secondary structure elements can be phenotypically suppressed by increasing divalent cation concentration. For

example, increasing Mg^{2+} concentration from 2 mM to 10 mM suppresses loss of splicing activity in *Tetrahymena* LSU mutants in which P7 is disrupted¹². This effect has been attributed to Mg^{2+} stabilizing the interactions that are mutationally weakened. But the opposite effect was found for the mutations destabilizing P9.0. We performed self-splicing experiments at 0, 2, 5 and 10 mM $MgCl_2$ (Fig. 3). Higher Mg^{2+} concentrations did not increase exon ligation activity for the core and 3'-splice-site mutants. Instead, the ratio of spliced-to-free exons decreased with increasing Mg^{2+} concentrations. Lower divalent cation concentrations favour exon ligation in splicing of the wild-type and compensatory mutant preRNAs as well. These results and phylogenetic data⁶ indicate that P9.0 could be unstable or transient, or both. It is possible that P9.0 forms only after guanosine attack on the 5' splice site.

We assayed the mutant and wild-type preRNAs for activity in *trans*-splicing and 3'-splice-site hydrolysis assays (ref. 4, and data not shown). The core and 3'-splice-site mutations resulted in loss of activity in both of these assays. Activity was restored to nearly wild-type levels by the compensatory mutation. It is interesting that preRNAs containing mutations that disrupt P9.0 show efficient 3'-splice-site cleavage in the presence of guanosine (Figs 2 and 3), but lack activity in the absence of the nucleotide. Although the reason for this effect is unknown, binding of

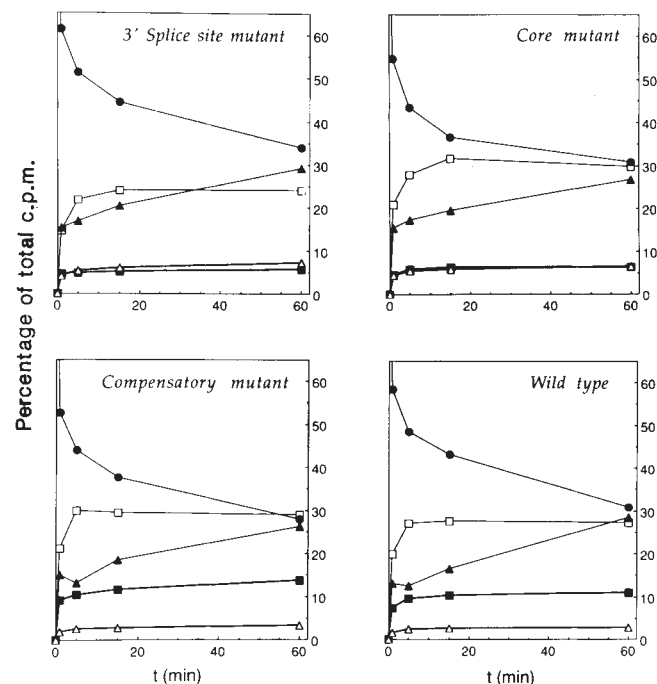


FIG. 2 Time course of self-splicing reactions. Mutants in *Tetrahymena* LSU were constructed by oligonucleotide-directed mutagenesis of phagemid pTZIVSU as described⁹. Plasmids were linearized by digestion with restriction endonuclease *Bgl*I. Radiolabelled preRNAs were synthesized by transcription with bacteriophage T7 RNA polymerase in the presence of [α -³²P]UTP as described⁹, purified by denaturing polyacrylamide gel electrophoresis, and heat-renatured (S. Walstrum and O. Uhlenbeck, personal communication). Self-splicing reactions were performed at 42 °C in a buffer containing 200 μ M GTP, 10 mM $MgCl_2$, 30 mM Tris-HCl, pH 7.5, and 100 mM NaCl. Reactions were stopped at the indicated times by pipetting into excess EDTA solutions on ice, and separated by electrophoresis on a 4% polyacrylamide-urea gel. Results were quantitated using a Betagen radioanalytic imaging instrument. The 3' splice-site mutant, G313 $\rightarrow$ U and A314 $\rightarrow$ U; core mutant, U412 $\rightarrow$ A and C413 $\rightarrow$ A; compensatory mutant, G313 $\rightarrow$ U, A314 $\rightarrow$ U, U412 $\rightarrow$ A and C413 $\rightarrow$ A; wild type, pTZIVSU; ■, spliced exons; □, unspliced 3' exons (177 nucleotides); ●, preRNA; ○, circular IVS (intervening sequence) species; ▲, linear IVS species. At $t=0$, 100% of total counts were in preRNA. Free 5' exons were visualized on the gels, but were not quantitated because they contain little radioactivity owing to their short length (27 nucleotides).

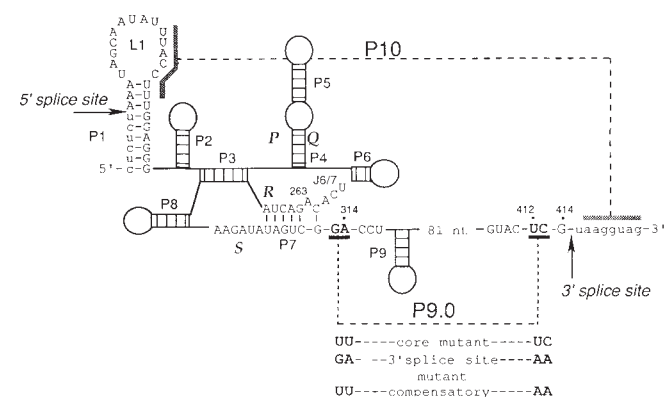


FIG. 1 3' Splice site-binding sequence in the catalytic core of *Tetrahymena* LSU. An abbreviated structural model is shown that includes all secondary structure elements common to group I introns (P1-P10). Sequences shown correspond to *Tetrahymena* LSU. P9.0 indicates base-pairing interaction between 3' splice site and core demonstrated by mutational work described here. Sequences of mutants constructed are shown. Upper-case letters, intron sequences; lower-case letters, exon sequences; P, Q, R and S, conserved sequence blocks in the catalytic core of group I introns; J6/7, sequence segment joining P6 and P7. nt, Nucleotide.

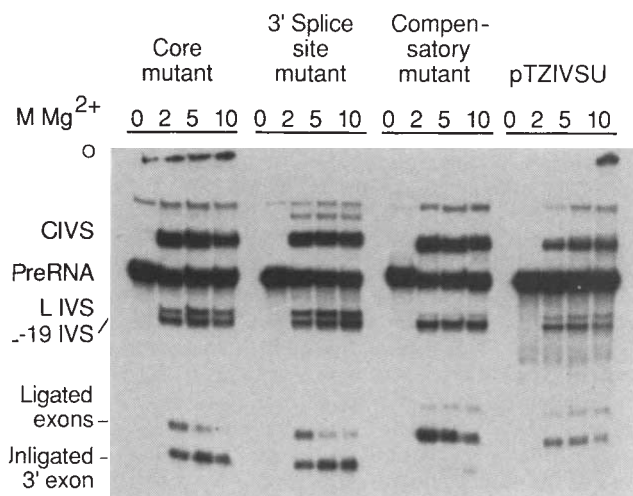


FIG. 3 Mg^{2+} dependence of self-splicing reactions. Splicing reactions were carried out for 1 min as described in the legend to Fig. 2, except that the $MgCl_2$ concentration was varied as indicated. C IVS, circular IVS species; L IVS and L-19 IVS, linear IVS species. The minor bands migrating above C IVS and above ligated exons have not been characterized. Free 3' exons were identified by comparison with CpU-3' exon obtained by *trans*-splicing of wild-type preRNA (not shown). Free 5' exons were visualized on this gel for core and 3'-splice-site mutants only, but were not present on the segment of the gel shown here.

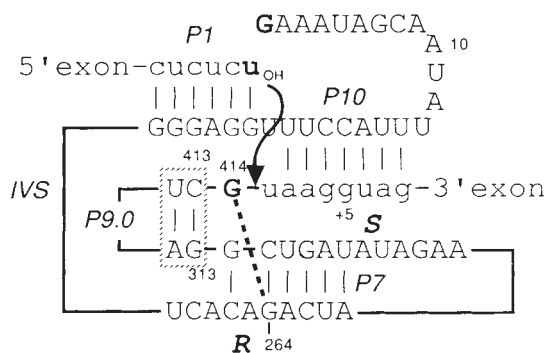


FIG. 4 Tripartite model for 3'-splice-site selection in group I introns. Model for secondary structure at the exon ligation step of splicing is shown. Interactions believed to be important for 3'-splice-site selection⁶ are indicated (P9.0, P10 and G264-G414). G264 is the binding site for guanosine mononucleotide in the first step of splicing, and for G414 in the exon ligation step¹⁴ (dotted line).

guanosine could stabilize the active site so that 3' splice sites can be efficiently recognized and cleaved in the absence of P9.0.

Primer extension and dideoxy chain termination sequencing experiments indicate that these mutations do not affect the choice of 3' splice site (data not shown). Therefore, although P9.0 is important for exon ligation, it cannot solely be responsible for 3'-splice-site selection. Other structural features of the P9.0[5'] and P9.0[3'] mutant preRNAs must specify the 3' splice site. Our model for 3'-splice-site selection⁶ invokes three different interactions—P9.0, P10 and a binding site for the intron terminal G (Fig. 4). P9.0 and P10 together are likely to align the 3' splice site for attack by the 5' exon^{6,8,13–14}. A binding site for the intron-terminal G activates the splice site for efficient attack¹⁵. Similar to our findings for P9.0, neither genetic disruption of P10^{13,16}, nor mutation of G414¹⁵ affects 3'-splice-site choice, although the level of activity is greatly affected in the G414 mutants. Therefore it is likely that accurate 3'-splice-site recognition can be accomplished by two of the three interactions described above⁶. Genetic work to test this hypothesis is in progress.

These results establish the existence of a binding sequence for the 3' splice site in the catalytic core of the self-splicing *Tetrahymena* LSU intron. A mutation in the catalytic core of the ribozyme specifically inhibits the exon ligation step of splicing, whereas a complementary second-site mutant at the 3' splice site restores that activity. This work provides important insights into the three-dimensional structure of the ribozyme active site. These results indicate that sequences at the 3' splice site are orientated as shown in Fig. 4, and that the universal G at the 3' splice site could be bound to a site near or in the highly conserved segment of P7 proximal to P9[5']. Recently, the G-binding site has been identified as the universal G264-C311 pair in P7 (ref. 14).

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Articles may contain a few subheadings of two or three words. The meaning of the text should not depend on the subheadings, whose function is to break up the text and to point to what follows. There should be fewer than 50 references.

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Commentary articles deal with issues in, or arising from, research that are also of interest to readers outside research. Some are commissioned, most are unsolicited. They are normally between one and four pages of *Nature* in length.

News and Views articles are intended to inform non-specialist readers about a recently published advance. Suggestions should be made to the News and Views Editor. Illustrations are welcome. Proposals for meeting reports should be agreed in advance.

Scientific Correspondence is for the discussion of scientific matters, including contributions published in *Nature*. Priority is given to contributions of less than 500 words and five references. Figures are welcome.

Submission. All manuscripts may be submitted to either London or Washington. They should not be addressed to editors by name. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value \$5' to prevent the imposition of import duty and value-added tax (VAT).

GENERAL

All contributions submitted for publication in *Nature* should conform with these rules.

Manuscripts should be typed, double-spaced, with a good-quality printer, on one side of the paper only. Four copies are required, each accompanied by lettered artwork. Four copies of half-tones should be included. Reference lists, figure legends and so on should be on separate sheets, all of which should be double-spaced and numbered. Copies of relevant manuscripts in press or submitted for publication elsewhere should be included, clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their reference numbers.

Titles should say what the paper is about with the minimum of technical terminology and in fewer than 80 characters. Authors should avoid active verbs, numerical values, abbreviations and punctuation and should include one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

Colour Artwork. A charge of £500 a page is made for 4-colour figures. Inability to meet these costs will not prevent the publication of essential colour figures if the circumstances are explained.

Figure legends must not exceed 300 words and ideally should be much shorter, and should use telegraphic form wherever possible. The figure should be described first, then, briefly, the method. Reference to a method described elsewhere is preferable to a full description. Methods should not be described in detail in the text.

References should be numbered sequentially as they appear in the text, followed by those in tables and finally those in the figure legends. Reference numbers apply only to papers published or in the press, and a different number should be given to each paper cited. All other forms of reference (including unnumbered abstracts) should be included in the text as personal communication, manuscript in preparation or preprint (with number and institution where appropriate). Text should not be included in the reference list. References should be abbreviated according to the *World List of Scientific Periodicals* (fourth edition, Butterworth, London, 1963–65). The first and last page numbers should be cited. Reference to books should clearly indicate the publisher and the date and place of publication.

Abbreviations, symbols, units and Greek letters should be identified the first time they are used. Acronyms should be avoided as much as possible and, when used, defined. Editors will shorten words if necessary. In case of doubt, SI units should be used.

Footnotes should not be used except for changed addresses or to identify the corresponding author if different from the first-named.

Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.

EXAFS: a probe for metalloproteins

G.P. Diakun

X-ray absorption spectroscopy provides a method for studying the local environment around a metal atom in a protein.

THE EXAFS (extended X-ray absorption fine structure) technique is used to probe the immediate environment of a specific type of atom in biological, chemical and physical systems. Thus, it is capable of providing information on the number, type and distance of ligand atoms from a metal atom in a metalloprotein. Features of the technique that make it particularly suitable for biological systems are: (1) measurements can be performed on any state of matter; (2) in principle, any element may be probed; (3) accuracy in the determination of metal-ligand distances is very high; and (4) the theoretical basis in terms of electron scattering is well defined.

The limitations of the EXAFS technique include: (1) a lower limit of concentration of approximately 1–2 mM for 3d metals and ~0.5 mM for 4d metals; (2) the EXAFS of an atom occupying several different sites will give an average of all the sites; (3) co-ordination numbers can be imprecise because of the high correlation between the number of atoms and their Debye-Waller-type factor; (4) atoms of a similar atomic weight (for example, oxygen and nitrogen) cannot be distinguished from one another; and (5) it is possible that a ligand may be missed because of its weaker contribution in the presence of the primary scatterer. However, if care is taken during the analysis, the technique offers an excellent means of structural characterization of the local environment of metal sites in biological systems.

EXAFS spectra

A typical X-ray absorption spectrum is shown in Fig. 1, this may be divided into three parts. First, the pre-edge region, which decreases monotonically with increasing photon energy. Second, the edge region also known as XANES (X-ray absorption near-edge structure), which contains bound state transitions and reflects co-ordination geometry, symmetry of unoccupied atomic states and effective charge on the absorbing atom. Third, EXAFS, which extends from ~30 to 1,000 eV above the absorption edge and consists of an oscillatory variation of the X-ray absorption cross-section as a function of energy. This may be measured either directly in a transmission experiment or indirectly via the products of absorption, for example fluorescent radiation. Because of the low concentration of metal centres in most metallopro-

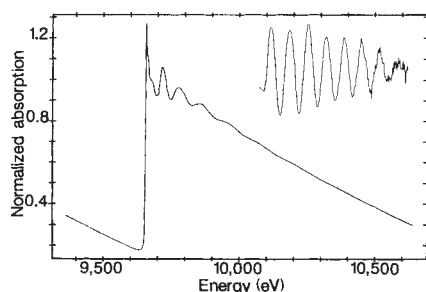


FIG. 1 An X-ray absorption spectrum at the zinc K-edge. The inset is the EXAFS with the background removed. The ordinate has been multiplied by the cube of the abscissa to enhance the EXAFS at higher energies.

teins, the latter is the preferred mode of detection.

Behind the theory

Although this phenomenon has been known for over 50 years, it was not until the 1970s that any great interest was shown. This was due to the development of a single scattering short-range order theory¹ which gave good agreement between theory and experiment from approximately 50 eV above the absorption threshold. The advent of synchrotron radiation brought a great improvement in both the speed of data collection and the quality of data that could be obtained². At the same time, theoreticians^{3,4} developed the modern EXAFS theory, which allows a single scattering formalism (called 'curved wave') to describe the observed data, although most EXAFS studies have used a simplified version termed 'plane wave'. The more exact theory^{3,4} is mathematically complex and a great deal of computational time is required to calculate a theoretical spectrum. Recently, this has been simplified⁵ without compromising its exact nature for polycrystalline and amorphous samples, and has reduced the computational time to that of the 'plane-wave' calculation.

Early EXAFS studies

The first EXAFS study of a biological system examined the iron K-edge EXAFS of the iron-sulphur protein rubredoxin⁶. At the time of this study, a crystallographic structure was available⁷ which suggested that one Fe-S bond was extremely short 1.95–2.05 Å as compared with the three other Fe-S bonds at 2.3 Å. Interpretation of the EXAFS data showed conclusively that all the Fe-S bonds were

equivalent at $2.26 \text{ Å} \pm 0.01 \text{ Å}$. Re-interpretation of the crystallographic data has brought the two techniques into agreement⁸. This shows the complementarity of the two structural techniques. The reduced protein was also investigated by EXAFS and showed an increase in the Fe-S distance of 0.05 Å over the oxidized form. No other physical technique at present is likely to have produced the same information with any certainty.

Zinc environment

An area where EXAFS can make a substantial contribution is in the study of the zinc environment in metalloproteins. As zinc has a filled 3d shell, it is not accessible by spectroscopic techniques such as optical absorption and electron paramagnetic resonance.

To resolve this problem, substitution of the zinc by a more spectroscopically active metal, such as cobalt or cadmium, has been carried out. It should be noted that these alternative metal ions do not always represent the true environment of the zinc atom in a protein. This has been elegantly demonstrated in the EXAFS study on phospholipase C⁹. In this protein there are two zinc sites, one structural the other catalytic. It is feasible to replace the zinc atom in the catalytic site by cobalt. Analysis of the EXAFS of both metals in this site reveals that the co-ordination number is greater for the cobalt ion than for zinc. This clearly shows that the cobalt spectroscopic results cannot be extrapolated to the native zinc site, and emphasizes the importance of EXAFS as a direct probe of zinc co-ordination in metalloproteins.

Multiple scattering

The enzyme superoxide dismutase has been studied extensively using EXAFS. It has a molecular weight of 32 kDa and contains two subunits, each with one copper and one zinc atom. The Cu(II)-Zn(II) are separated by ~6 Å and bridged by an imidazole ring from a histidine residue. It was hypothesised that when the enzyme was reduced the bridging imidazole became deprotonated and moved away from the copper atom. EXAFS studies of the copper site in the oxidized and reduced protein showed a reduction in co-ordination from four imidazole groups to three around the metal atom¹⁰. The zinc EXAFS and XANES showed virtually no change, suggesting it was not the bridging imidazole group that was deprotonated. Unfor-

tunately, it is impossible to identify which of the other three groups around the copper atom it might be.

What this study did highlight was the inaccuracy in the bond distance of the outer carbon and nitrogen atoms in the imidazole ring, they were foreshortened by $\sim 0.3\text{--}0.4$ Å. Primarily, EXAFS results from the single scattering of the photoelectron. Exceptions occur where three or more atoms are arranged co-linearly or approximately so, and multiple scattering of the electron can take place. This provides a contribution to the EXAFS spectrum, as in the case of the outer shells of the imidazole ring. Examples of other ligands that produce this effect are porphyrin, tyrosine, and the inhibitors cyanide and azide. This was the driving force for introducing the multiple scattering formalism⁴ into the analysis package at Daresbury.

Future prospects

In future, multi-element energy-dispersive detectors will be available¹¹, which will allow high quality EXAFS data to be collected on 0.5 mM 3d metals in proteins. In conjunction with developments in detector technology, new storage rings are being built and improvements are being made in monochromator design, which will open up a whole new range of possible protein structure studies. Examining intermediates looks promising, with progress being made in time-resolved¹² and freeze-quench techniques¹³. Finally, advances in analytical procedures¹⁴ should make the treatment of multiple scattering effects routine, and interpretation of the XANES combined with the EXAFS will provide geometrical as well as radial information about a metal centre. □

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Analytical aids

With over 800 exhibitors, next week's Pittsburgh Conference in New York will unveil the latest and the best in spectroscopic and analytical instrumentation: an electrophoretic light-scattering spectrophotometer, a biocompatible HPLC system and much more.

For the rapid concentration of samples, Zymark recommends the automated TurboVap **evaporator workstation** (*Reader Service No. 101*). The TurboVap concentrates samples up to ten times faster than Kuderna-Danish, hot block, rotary evaporators and nitrogen blow-down methods, and with more reproducible and comparable recovery rates, says Zymark. TurboVap can automatically



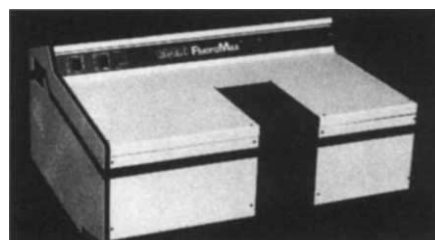
Zymark's TurboVap evaporator for rapid sample concentration.

and independently control the evaporation of each sample, perform an optional solvent exchange and stop the process at a pre-set endpoint. The evaporator comes in two sizes, each accommodating six samples: the 50-ml and 200-ml evaporators for samples of less than 50 ml and less than 200 ml, respectively. Each instrument has an integral water bath which controls the thermal conditions, limiting, says Zymark, the risk of semivolatile analyte loss. At the conference, Zymark will be bringing out a small-volume evaporator — a TurboVap LV 25-ml version that can hold 1-50 samples. See Zymark's full range of evaporators in booth 6004.

Dionex has launched a new line of **anion exchange columns** called ProPac PA1 designed for separating proteins and nucleic acids (*Reader Service No. 102*). Using ProPac PA1 columns, ion exchange separation with resolutions approaching reversed-phase separations can be achieved, says Dionex. The columns incorporate patented MicroBead resin technology where 0.2 micron resin par-

ticles are bound to a polymeric substrate. This configuration allows much faster mass transfer and results in high-resolution separations and short separation times. Typical flow rates are 0.1-3.0 ml min⁻¹ (4 mm ID). Columns are stable over the full pH range, says the company. ProPac PA1 columns come in three sizes: 4 × 50 mm for fast protein analysis, 4 × 250 mm for standard analytical separations, and 9 × 250 mm for semi-preparative applications, providing a five-fold increase in capacity. Dionex will be in booth 5520.

Spex Industries will be introducing the FluoroMax **spectrofluorometer** at the exhibition, which Spex will be offering as a complete automated package that includes a PC-compatible computer (*Reader Service No. 103*). The \$25,995 (US) FluoroMax contains all reflective optics for focusing at all wavelengths and a photon-counting detector for improved sensitivity, says Spex. The spectrofluorometer has a scan speed of 160 nm sec⁻¹ and time-based measurements can be as rapid as one millisecond per data point. For ease of use, FluoroMax is under full PC control with menu-driven DM3000F software. In

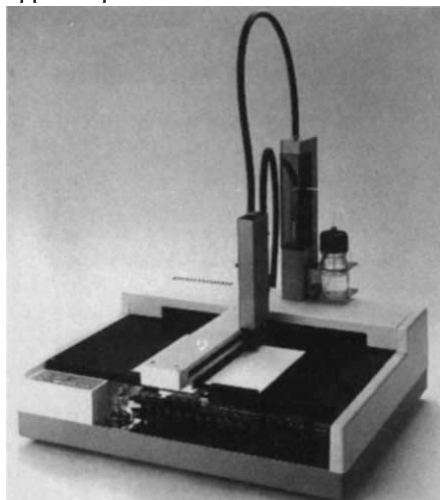


High-tech optics and a photon-counting detector: features of FluoroMax.

addition, it has automated variable slits, self-calibration for quick start up, and fully corrected excitation (200-900 nm, optimized in UV) and emission (200-900 nm, optimized in visible) spectra. See the FluoroMax in action in booth 5018.

This April, Camag of Switzerland is making available an **automatic sampler for thin-layer chromatography (TLC)** — Sampler III (*Reader Service No. 104*). The instrument consists of two independent pumps: a small precision pump for sample dosage and a larger pump for quick rinsing of the system. The Sampler III is computer-controlled and can use the same IBM computer or compatible that also controls the Camag TLC scanner II for densito-

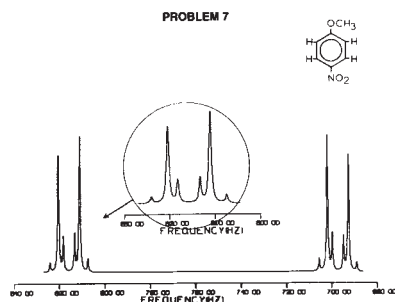
metric measurements — hence, all procedures from sample application to data evaluation can be under computer control, says Camag. Samples can be applied spotwise or as a narrow band on



Camag's automated TLC sampler for spot and band sample application.

plates or sheets measuring up to 200 × 200 mm. Users can programme sample positioning for normal development, development from both sides, for circular and anticircular chromatography. The volume range for spotwise or band application is 50 nl to 5 µl and 1 µl to 20 µl, respectively. The sample application head makes lateral movements at a speed of 250 mm sec⁻¹. Sample dispensing speeds can be selected for each chromatogram. See Camag in booth 1941.

Savant Audiovisuals has introduced three new **NMR audiovisual training programs** (Reader Service No. 105). The NMR Spectral Interpretation program comes in two parts: Part 1 — NMR-2000 — presents the most important proton absorbances and uses illustrative NMR spectra that exhibit specific spectral



Audio-visual aids for NMR from Savant.

characteristics. Part 2 — NMR-3000 — complements NMR-2000 but assumes that the user is already familiar with basic NMR. NMR-3000 is designed to help recognise and analyse the more complex spin-spin splitting patterns found in most proton magnetic resonance spectra. Both programs are interspersed with spectral problems, which are reviewed and discussed in detail.

The ¹³C NMR Spectral Interpretation program — NMR-4000 — covers shielding constants, chemical shifts, sign conventions, additivity methods, decoupling methods and isotopic substitution (²H labelling) techniques. NMR-2000 runs for 82 minutes and is available on slide tape or video, while NMR-3000 and NMR-4000 run for 79 and 37 minutes, respectively, and are available only on slide tape. See Savant's full line of audiovisual aids in booth 3262.

IC sample clean-up

Anotop ion chromatography disposable syringe filters for preparing samples intended for IC analysis are new from Anotec Separations (Reader Service No. 106). The IC filters provide low levels of leachable ion species, says Anotec. Typically, average leaching levels are: fluoride 10 p.p.b., chloride 15 p.p.b., bromide 20 p.p.b., sulphate 30 p.p.b., phosphate 75 p.p.b., nitrite 30 p.p.b. and nitrate 30 p.p.b. Anotop IC filters contain a 10-mm disc of Anopore inorganic membrane in a polypropylene housing. Additional features include wide solvent compatibility of the filters, and a naturally hydrophilic surface that eliminates the need for binders, wetting agents, glues, solvents or plasticisers. Anotec's product line will be on show in booth 5757.

Eliminate the need for pre-flushing in ion chromatography procedures by pre-filtering samples with the new 13 mm Acrodisc syringe filters for IC, says Gelman Sciences (Reader Service No. 107). Acrodisc filters ensure that the



Keep leachable ion species low with IC filters from Gelman.

sample has inorganic extractable levels for chloride, sulphate and nitrate that are less than 0.05 p.p.m., says Gelman. These latest IC filters will be among the many microfiltration products that will be on display in booth 4519.

HPLC systems

At Pittcon, SepTech will be launching its latest **semi-preparative HPLC system** — the biocompatible Novaprep 5000 (Reader Service No. 108). This totally inert HPLC system is designed for high-efficiency preparative chromatography of

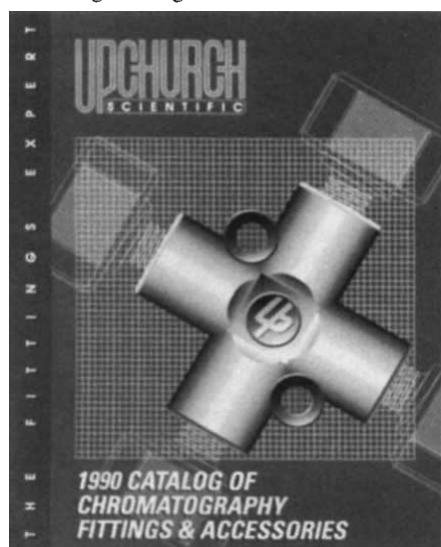
metal-sensitive biomolecules such as recombinant proteins and peptides, monoclonal antibodies and enzymes. Bio-Novaprep 5000 provides flow rates of between 1.0 ml min⁻¹ to 140 ml min⁻¹, without, says SepTech, the need for changes of the pumphead or helium sparging. A maximum operating pressure of 5,000 p.s.i. across the flow-rate range allows the use of high-efficiency packing media. Detection is provided by a variable UV/visible (190-600 nm) or RI (split stream) detector. Optional TurboPrep HPLC automation software provides automation of sample injection, continuous gradient formation, fraction collection, as well as interactive methods development for easier scale-up. See the Bio-Novaprep 5000 HPLC set up in booth 1009.

To avoid the problems associated with tetrahydrofuran (THF) attacking CTFE components and fittings found in standard HPLC mobile-phase handling systems, Kontes has come up with a **THF-resistant mobile-phase handling system** — the Ultra-Ware reservoir (Reader Service No. 109). The Ultra-Ware reservoir replaces CTFE with 316 stainless steel and is designed for filtration, degassing, storage and delivery of THF mobile phases. Kontes says, THF can be stored in Ultra-Ware reservoirs for days or weeks, without the need for repurification, as the reservoirs are coated with an autoclavable UV-absorbing plastic. Conical-bottomed THF-resistant reservoirs come in sizes ranging from 250 ml to 20 litres. Prices range from \$480-1,368 (US). Visit booth 4244 and see Kontes' latest scientific instruments.

For HPLC users interested in saving solvent and increasing detector sensitivity, Supelco has introduced four new 1.0-mm ID **microbore HPLC columns** for LC/MS applications (Reader Service No. 110). The 30 cm × 1.0 mm microbore columns are packed with 5 µm spherical silica with a pore size of 100 Å. Typically, flow rates can range between 10-50 µl min⁻¹ with injection volumes of 0.1-1.0 µl. The four types of column differ only in their bonded phase: none (pure silica); C-18, Octadecylmethylsilyl; deactivated C-18, Octadecylmethylsilyl; and C-8, Octyldimethylsilyl. Each is priced at \$288 (US). See Supelco and this new line of columns in booth 4541.

For all users of HPLC instrumentation, Upchurch Scientific has issued its free **1990 full-colour catalogue** of fittings and accessories for HPLC (Reader Service No. 111). The 144-page catalogue contains a detailed technical reference section to help HPLC users understand the range of fittings available for HPLC instruments. Other sections cover solvent compati-

bility, material properties, systems applications, and recommendations for choosing fittings most suited to user



Reading matter from Upchurch Scientific.

requirements. Upchurch's catalogue also highlights the company's wide range of PEEK tubing IDs, biocompatible filters, frits and check valves. See the company's latest accessories for HPLC in booth 1909.

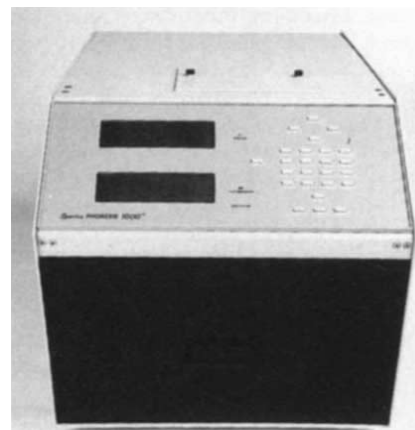
Capillary electrophoresis

Waters Chromatography Division of Millipore will be exhibiting, in booth 5132, the Quanta 4000 **capillary electrophoresis system** for the separation of inorganic ions, small organic ions, pharmaceuticals, peptides, proteins, carbohydrates and DNA samples (*Reader Service No. 112*). The fully automated



Quanta 4000 system is designed for multiple unattended analyses and has an autopurge facility that cleans the capillary through after each run. The system offers both automatic hydrostatic and electromigration injection procedures — the former uses gravity for sample injection while the latter uses high voltage, which, Waters says, is particularly useful for gel-filled capillaries. Quanta 4000 is fully compatible with Waters' data workstations and features a high-sensitivity, low noise UV/visible detector for trace-level detection. Injection time, run time, sample voltage, and run voltage can all be pre-programmed. The Quanta 4000 can be configured to collect fractions for further analyses.

Spectra PHORESIS 1000 is the new **automated capillary electrophoresis system** from the Autolab Division of Spectra-Physics, which will be on display in booth 3317 (*Reader Service No. 113*). The \$35,000 (US) Spectra PHORESIS 1000 system uses fibre optics to detach the optical sensor from the detector body. This set up allows independent thermostating of the entire capillary, says Spectra-Physics, which improves reproducibility. Three detection modes are available: single-wavelength mode for p.p.m. sensitivity in the detection of peptides; multi-wavelength mode for the



Spectra-Physics' Spectra PHORESIS 1000 HPCE system: see booth 3317.

simultaneous monitoring of several wavelengths; and high-speed scanning mode for producing continuous spectra for peak identification and the detection of trace impurities. When used in conjunction with an optional IBM PS/2 computer controller and data station that uses Presentation Manager, three-dimensional plots — absorbance, wavelength and time — can be generated. For unattended overnight operation, the system's autosampler can accommodate up to 80 samples.

GC, SFC and SFE

Programmable pressure values, a wide flow-rate range and multi-pump control are all features of the new Model 260D **syringe pump for supercritical fluid extraction and chromatography (SFE/SFC)**, says Isco (*Reader Service No. 114*). When using the Model 260D, fluid rates can be set to between 1 $\mu\text{l min}^{-1}$ to 40 ml min^{-1} and at pressures up to 7,500 p.s.i. The SFE/SFC pump features a function-based keypad with LCD. A single controller can operate up to three pumps simultaneously, says Isco, which is useful for modifier addition or for alternate refilling and delivery, providing continuous flow without limitations of volume. The Model 260D has a 260-ml capacity and takes less than three minutes to refill. For high-flow applications or cooling purposes, the pump cylinder can be fitted with an optional temperature-control jacket. Optional accessories include SFE kits with

quick-load chambers to accommodate a wide range of solid and liquid samples, and connection packages that can connect most GCs for SFC. The pump and many other instruments will be on show in Isco's booth, number 4153.

Sievers Research announces its latest **sulphur-chemiluminescence detector (SCD)** — the Model 350B — which is designed for gas chromatography (GC) and supercritical fluid chromatography (SFC) (*Reader Service No. 115*). Based on the earlier Model 350, the Model 350B incorporates new electronics which provide a wider linear range of six orders of magnitude, reduced background noise and no measurable peak broadening, says Sievers. The detector can be retrofitted to any GC or SFC that has a flame ionization detector (FID). With this configuration, the sulphur signal from the SCD Model 350B and hydrocarbon signal from the FID can be monitored simultaneously, without the need for post-column splitting. The Model 350B has a less than five-picogram sensitivity and can be used with either packed or capillary columns. See Sievers in booth 3215.

AA spectroscopy

Visitors to booth 3068, can see Perkin-Elmer's **flow injection system** — the FIAS-200 — for atomic absorption (AA) analyses (*Reader Service No. 116*). The FIAS-200 consists of two multi-channel peristaltic pumps for sample handling, diluents, and reagents for mercury/hydride and flame AA analyses. When used in conjunction with Perkin-Elmer's AS-90 autosampler and specific AA instruments, the company says, that FIAS-200 provides a fully automated



Perkin Elmer's FIAS-200 flow injection system — sample handling solutions for AA analyses.

sample handling system that is faster than conventional continuous-flow systems. FIAS-200 provides automatic sample dilution, reagent addition, and analyte pre-concentration. All these procedures are controlled from the system's keyboard. Analyses can be performed on samples of less than 1 μl and on concentrated solutions, which, Perkin-Elmer points out, would clog conventional systems. When configured as a mercury/hydride system, the FIAS-200 can perform up to 180 determinations per hour using minimal sample and reagent volumes.

ADVERTISEMENTS

For fast, sequential multi-element analyses of a single sample, previously possible only with inductively-coupled plasma systems, Analyte Corporation recommends the FS800 **atomic absorption spectrophotometer** (*Reader Service No. 117*). The \$45,000 (US) FS800 is designed to determine the concentration of up to 12 elements in a single liquid sample at a rate of up to three seconds per element, says Analyte. The AA spectrophotometer retains the benefits of flame AA, including freedom from spectral interferences and the ability to analyse concentrated samples and organic solutions. The system is supplied with a range of accessories, including a furnace, autosampler for liquids and hydride generator. The latter can convert sample elements to gaseous hydrides for the determination of mercury, and low-level traces of arsenic, bismuth, antimony, selenium, tin and tellurium in rapid sequence. The FS800 can be upgraded to an SL-16 for the sequential determination of 24 elements in a liquid sample. See the FS800 in booth 1027.

Users of Varian's SpectAA atomic absorption (AA) spectrometers may be interested in the company's latest **dedicated software** called Quality Control Protocols (QCP) (*Reader Service No. 118*). The \$500 (US) QCP program offers complete quality control over graphite furnace AA data. It automatically monitors check standards, blanks, spikes, matrix spikes and duplicates. The replicate percentage RSD feature automatically ensures that as many results as possible meet precision requirements without wasting analysis time. The overrange volume reduction facility reduces the programmed sample volume for all solution results outside the calibration range, repeating the procedure until the result is within range. This feature is designed to improve productivity and allow unattended operation by eliminating the need for manual sample dilution, says Varian. During the run, QCP provides hard-copy output of results where the user defines the amount of information in the report. Data can be exported to ASCII files, word-processing programs or databases. Visit Varian in booth 4736.

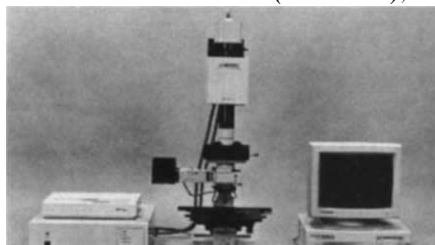
Specs, specs, specs

PL Separation Sciences, a Division of Polymer Laboratories, is launching its ELS-800 Otsuka **electrophoretic light-scattering spectrophotometer** at Pittcon (*Reader Service No. 119*). The \$44,000 (US) ELS-800 system (not including PC/AT or PS/2-type computer) has been developed for the measurement of mobility, zeta-potential distribution, and size distributions of fine particles and macromolecules. PL Separation Sciences says the instrument will provide valuable

information for studies of surface characterization, interactions and surface modifications. See the new ELS-800 in booth 1004.

Applied Research Laboratories (ARL) will be putting Fisons Instruments' SpectraSpan 7, a **multi-element spectrometer**, through its paces at the Pittsburgh Conference (*Reader Service No. 120*). SpectraSpan 7 is a benchtop spectrometer, which is offered in either simultaneous or sequential configurations. Fisons says, it can determine up to 24 elements simultaneously in 30 seconds. Designed for use in a clinical setting where it can analyse a wide variety of samples, SpectraSpan 7 features push-button controls and state-of-the-art electronic circuitry for enhanced real-time data acquisition, including automatic background subtraction. As an accompanying package, the IBM PS/2-based Plasma-Vision software provides a powerful tool for analytical analysis, says Jasco. It features pull-down menus, colour graphics and on-line prompts. Plasma-Vision supports full automatic operation, including a random-access autosampler, profiling, calibration, normalisation and analysis.

Hitachi Instruments, exhibiting in booth 3137, has introduced a new Fourier transform, visible microscope **photodiode array spectrometer** the U-6000 (*Reader Service No. 121*). The \$100,000 (US) U-6000 is designed to measure the transmittance, specular reflectance (bright field) and diffuse reflectance (dark field), or



Under the microscope at Pittcon: Hitachi's photodiode array spectrometer.

fluorescence of microscope samples using a standard microscope and interferometer with diode array detector. The instrument's spectral range is 400-840 nm. Hitachi says, typical applications for the U-6000 system, which can measure particles as small as three microns in diameter spectrophotometrically, include forensic analysis, the analysis of biological cells and tissues, and wafers (semiconductors).

Jasco has launched a new **laser-Raman spectrometer** — the NR-1100 — which resolves close peaks for the identification of liquids, gases and solids (*Reader Service No. 122*). The \$104,000 (US) NR-1100 spectrometer features a double monochromator and dual detector system for high- and low-level scatter diagrams. The

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instrument scans in the range of 330-910 nm. Scattered light is processed through a one-metre double monochromator and then on to a detector system.



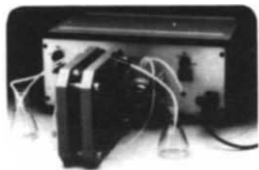
See Jasco's laser-Raman spectrometer in action in booth 1536.

Larger scattering signals that are typical of liquid samples usually require the d.c. amplifier system, says Jasco. The sample chamber in the NR-1100 is large enough to accommodate fixtures and holders as part of the set up. The space allows for heating, cooling and can hold large crystals. Optional extras include compatible sample holders, beamsplitter, polarised light filters, and multi-channel detector with a one-inch light-capture surface. The NR-1100 can be seen in booth 1536.

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Spectra-Tech, exhibiting in booth 4659, has brought out a new line of **FT-IR sampling accessories** for use with most spectrophotometers, including the Perkin-Elmer 1600 series, Bomem MB series, Mattson Galaxy series, Midac, Philips PU9800, Nicolet 205 and 500 series (*Reader Service No. 123*). The 'Baseline FT-IR sampling accessories' are designed to provide users with all the necessary tools to sample gels, pastes, liquids, powders and films. They are slide mounted for easy installation and are provided with pre-aligned optics. The



Spectra-Tech's 'Baseline family' of FT-IR sampling accessories.

'Baseline family' includes a horizontal ATR kit, diffuse reflectance kit, and a 30° specular reflectance accessory. Parts of the 'Baseline family' accessory kit can be purchased individually. Visit Spectra-Tech in booth 4659.

The French company Secomam has expanded its line of **UV/visible spectrophotometers** to include a scanning S-1000G (*Reader Service No. 124*). The S-1000G scans the entire spectral range between 200-1,000 nm and has a 1,200 line mm⁻¹ holographic grating and a 2-nm bandwidth which provides measurements up to 3 Abs. The cell compartment can hold a cell with a light path up to 100 mm. The instrument's microprocessor control provides automatic wavelength calibration, light-source testing, automatic lamp switching for different wavelength requirements, and commutation of second-order filters.

Mass spectroscopy

In booth 5420, Finnigan MAT will be showing off its latest **triple-stage quadrupole mass spectrometer** — the TSQ 700 GC or LC/MS/MS/DC system designed for both MS and MS/MS (*Reader Service No.*



The TSQ 700 mass spec from Finnigan MAT.

125). According to Finnigan MAT, the key to the system's performance lies in the non-linear octapole collision cell, which provides maximum transmission of ions under multiple-collision conditions, while preventing high-energy neutrals from reaching the detector. In addition, the 20-kV dynode detector has been designed to be virtually 'noise-free'. The TSQ 700 offers a variety of inlets and ionization techniques. Standard features include a gas chromatograph, switchable EI/CI ion source with exchangeable ion volumes and beam-collimating magnets, positive and negative ion detection on alternate scans, mass range of 10-4,000 Da, and a cradle vacuum system with differential turbomolecular pumping. At the heart of the TSQ 700 system, is the new X-windows-based version of ICIS data system running on a DECstation 2100 workstation with DEC's version of UNIX — ULTRIX-32 operating system. Finnigan MAT's Instrument Control Language allows the mass spectrometer to make real-time data-dependent decisions during acquisition.

Delsi-Nermag of France offers a compact **benchtop gas chromatograph/mass spectrometer** called Automass, which the company claims is the systems equivalent of the best quadrupole mass spectrometer (*Reader Service No. 126*).



Delsi-Nermag's benchtop GC/MS — Auto-mass.

Designed for routine analyses, Automass has self-calibration and automatic regulation features, which ensure hands-off operation. Automass provides picogram sensitivity with some compounds and is unaffected by residual traces of water vapour, says Delsi-Nermag. The system is modular which allows retrofit options — chemical ionization, negative ion detection systems or direct sample introduction. In addition, there are dedicated ion volumes for EI and CI with direct access for rapid sample changeover, and a differential pumping mode. See what else Delsi-Nermag has to offer the analytical chemist in booth 5554. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.